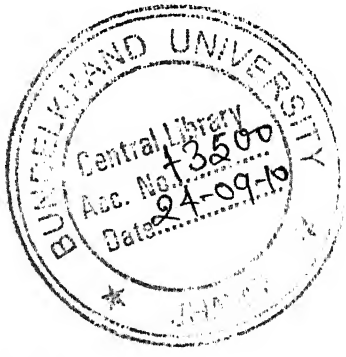
MOLECULAR MAPPING OF GENES CONFERRING DISEASE RESISTANCE IN URDBEAN, *VIGNA MUNGO* (L.) HEPPER USING RAPD AND AFLP MARKERS.

THESIS SUBMITTED FOR THE DEGREE

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY



By

PRAVAS RANJAN KOLE

DEPARTMENT OF BIOTECHNOLOGY

BUNDELKHAND UNIVERSITY JHANSI (U.P.)
INDIA

2009

CERTIFICATE

This is to certify that the research work done entitled "**Molecular mapping of genes conferring disease resistance in urdbean, *Vigna mungo* (L.) Hepper using RAPD and AFLP markers**" is submitted by Mr. Pravas Ranjan Kole under our guidance and supervision for the degree of Doctor of Philosophy in Biotechnology, at Bundelkhand University Jhansi (U.P) India. To the best of our knowledge and belief the thesis embodies the work of the candidate herself. It has been duly completed and fulfils all the requirements of the ordinance relating to the PhD degree of the university. This thesis is upto the standard both in respect of contents and language for being referred to the examiner.

Signature of the Supervisor

Dr. Jose Mathew,
LECTURER Department of Biotechnology
Bundelkhand University, Jhansi

Signature of the Co- supervisor

16/6/09

Dr. K.V. BHAT
Principal Scientist
NRC on DNA Fingerprinting
National Bureau of Plant Genetic Re
Pusa Campus, New Delhi-1100

Dr. K.V. Bhat
Principal Scientist, NRCDF
NBPGR, New Delhi

Dated : 18th June, 2009

Place : Jhansi

Contents

Acknowledgement

Gratitude cannot be seen or expressed. It can only be felt deep down in the heart and is beyond description. Although words are poor expressions of the debt of gratitude, one feels, these are all one has.

I owe my indebtedness and virtual veneration towards respected supervisor Dr. Jose Mathew, Professor, Deptt. Of Biotechnology, Bundelkhand University, Jhansi, for his constant inspiration, and generous guidance and constructive criticism during the course of this investigation and preparation of manuscript.

It is a great occasion for me to express my deep sentiments, legitimate regards and gratitude towards Dr. K.V. Bhat, Senior Scientist, N.R.C.D.N.A. Fingerprinting and my co-chairman, whose scientific acumen, meticulous guidance, unending enthusiasm and constant encouragement led me to complete this pain stating venture. I am indebted to him for the critical reviewing of the manuscript.

I record my profound thanks to Dr. (Prof) C.R.Kole, visiting professor Clemson University for his support and kind suggestions during the course of investigation.

I am extremely thankful to Dr. S.K. Sharma, Director, N.B.P.G.R., New Delhi for providing me all the facilities.

I also express my sincere thanks to the entire faculty members and scientists of N.B.P.G.R., New Delhi who imparted me the great wealth of knowledge, timely advice and support.

I am thankful to my senoirs Ruchi ma'm, Papiya ma'm, Vimla ma'm, and Sudeep sir,for their valuable advices and untiring supervision during my research work.

Heartfelt thanks to my college friends Shubhanjli , Mr Kumar Pranaw and Ms Pooja for being my good company, kind help and co-operation during the tenure of study.

I express my hearty thanks to my lab friends, Senthil, Pooja, Sunaina, Santosh, Sarwanan, Nupur, Niti, Nidhi, Nirupama, Avinash, Suraj and Praveen. I am also thankful to Rameshji, and Rajendarji for their help during the course of my investigation.

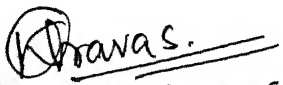
No words are enough to express my heartiest gratitude to my papa and mummy who have been the pillars of my strength, for having faith in me and encouraging me in all my endeavours. It's by their constant encouragement, affection, blessing and prayer that I have completed this Ph.D thesis work. I dedicate this small piece of work in their name.

I am deeply indebted to my sister, sonali for her support, my Jiju Dr. Pritam Nandi and Bhanja Priyanshu for their unconditional love help and blessing which enabled me to complete my Ph.D thesis work.

It was indeed a pleasant stay in the NBPGR for me and I once again thank all the members of NBPGR New Delhi.

Date : 18.June.2009

Place : New Delhi



(Pravas Ranjan Kole)

ABBREVIATIONS

%	-	Percentage
et al.	-	And others
AFLP	-	Amplified Fragment Length Polymorphism
CLS	-	Cercospora Leaf Spot
Fig	-	Figure
PCR	-	Polymerase Chain Reaction
RAPD	-	Random Amplified Polymorphic DNA
RFLP	-	Restriction Fragment Length Polymorphism
SSRs	-	Simple Sequence Repeats
STMS	-	Sequence Tagged Microsatellite site
STS	-	Sequence Tagged Site
NBPGR	-	National Bureau of Plant Genetic Resources
SRAP	-	Sequence Related Amplified Polymorphism

LIST OF TABLES

LIST OF FIGURES

Figure 18: Multiple Sequence Alignment for locus OLM1 using Clustal X (1.83) showing insertions and deletions of single nucleotide.

Figure 19: Multiple Sequence Alignment for locus SUSY using Clustal X (1.83) showing insertions and deletions of single nucleotide

Figure 20: Multiple Sequence Alignment for locus SHMT using Clustal X (1.83) showing insertions and deletions of single nucleotide

INTRODUCTION

1. INTRODUCTION

Pulses are unique crops that are a rich source of vegetable protein and have in-built mechanism to fix atmospheric nitrogen. India is a major pulse growing country in the world, with about 37% of area and 28% of the production. Pulses or food legumes are the most versatile and nutritious food available that includes beans, peas, and lentils. They are typically low in fats, contain no cholesterol and are high in proteins, potassium, iron and magnesium. They also have phytochemicals; a group of compounds that are known to help prevent chronic diseases such as cardiovascular diseases and cancers. In addition, they are a good source of fiber that can reduce the risk of developing diabetes and help lower blood cholesterol levels, thereby reducing the risk of heart diseases. Their by-products provide nutritious fodder to livestock. Because of their ability to fix atmospheric nitrogen, they play a key role in maintaining soil fertility and ensuring sustainability of production system, particularly in low input, small-scale agriculture.). India is the largest producer, importer and consumer of the pulses in the world accounting for 25% of the global production, 15 % of the trade and 27% consumption, as sizeable population of the country still depends on the vegetarian diet to meet its protein requirement. The country produces variety of pulses including chickpea (40%), pigeon pea (18%), urd bean (11%), mung bean (9%), lentil (8.6%), field pea (5%) and others to the tune of 13 to 15 million tones from an area of 22-33 million hectare with an average yield of 600 to 650 kg/ha. Domestic production of pulses after its peak of 14.91 million tones in 2003-04 had declined to 13.13 million tones in 2004-05 and to 13.39 million tones in 2005-06 (Ministry of Agriculture) due to adverse climatic conditions prevalent in the major production zones. In the current year (2006-07), the production is estimated to increase marginally to 14.1 million tones. This still falls short of the domestic requirement of 17 million tones which is increasing consistently with growing population, rising income, value addition and market opportunities. The widening gap in demand and supply has led to soaring prices of pulses during past two years. Also, inclusion of pulses in future trading and limited availability in the international market has further fueled the prices in the prices.

Molecular Mapping

A population used for gene mapping is commonly called a mapping population. Mapping populations are usually obtained from controlled crosses. Decisions on selection of parents and mating design for development of mapping population and the type of markers used depend upon the objectives of experiments, availability of markers and the molecular map. The parents of mapping populations must have sufficient variation for the traits of interest at both the DNA sequence and the phenotype level. The variation at DNA level is essential to trace the recombination events. The more DNA sequence variation exists, the easier it is to find polymorphic informative makers. When the objective is to search for genes controlling a particular trait, genetic variation of trait between parents is important. If the parents are greatly different at phenotypic level for a trait, there is a reasonable chance that genetic variation exists between the parents, although uncontrolled environmental effects could create large phenotypic variation without any genetic basis for the effects. However, lack of phenotypic variation between parents does not mean that there is no genetic variation, as different sets of genes could result in same phenotype.

Selection of Parents for Developing Mapping Population

Selection of parents for developing mapping population is critical to successful map construction. Since a map's economic significance will depend upon marker-trait association, as many qualitatively inherited morphological traits as possible should be included in the genetic stocks chosen as parents for generating mapping population. Consideration must be given to the source of parents (adapted vs 1 exotic) used in developing mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses and generally yield greatly reduced linkage distances (Albine and Jones, 1987; Zamir and Tadmar, 1986). Wide crosses will usually provide segregating populations with a relatively large array of polymorphism when compared to progeny segregating in a narrow cross (adapted x adapted). To have significant value in crop improvement programme, a map

made from a wide cross must be collinear (i.e. order of loci similar) with map constructed using adapted parents.

Types of mapping populations

Different types of mapping populations that are often sed in linkage mapping are: (i) F2 population; (ii) F2 derived F3 (F2:F3) populations; (iii) Backcrosses; (iv) Doubled haploids (DHs); (v) Recombinant Inbred Lines (RILs); and (vi) Near-isogenic Lines (NILs). The characteristic features, merits and demerits of each of these populations are briefly presented below:

***F2 population*:**

- Produced by selfing or sib mating the individuals in segregating populations generated by crossing the selected parents.
- F2 individuals are products of single meiotic cycle
- Ratio expected for dominant marker is 3:1 and for codominant marker is 1:2:1

Blackgram or urdbean is one of the important grain legumes of India and Munhgbean yellow mosaic virus (MYMV) disease is one of the most devastating diseases. Munhgbean yellow mosaic virus (MYMV), a whitefly-transmitted bigeminvirus, causes disease in blackgram. This virus is assigned to the genus *Begomovirus* within the family *Geminiviridae.* Plant resistance is the most common and efficient method of MYMV control. In blackgram, two symptom types yellow mosic and necrotic mottle, can be distinguished (Nair and Nene, 1974). Other crops, including cowpea, develop similar symptoms as a result of infection with MYMV. The necrotic mottle is usually associated with resistance (varma et al. 1992).

Linkage map construction typically requires cosegregation analyses of hundreds of Mendelian loci, most of them molecular markers, using a relatively large number of plants from a population in linkage disequilibrium (usually F2, BC1, or similar progenies). The mapping effort is considerable, particularly when the objective is to obtain a high-density map or to incorporate a large number of functionally meaningful markers, such as those

based on expressed sequence tag (EST) sequences, or markers that are particularly suitable for breeding applications, such as simple-sequence repeat (SSR) markers, into an existing map. A strategy to improve the efficiency of mapping, named selective mapping, was proposed by Visionet al. (2000). It consists of a two-step process in which, first, a mapping population of usual size (N¼60–250) is used to construct a saturated framework map with markers placed on it with high precision, and second, new markers are added to this map with lower precision using a selected subset of highly informative plants. The final objective is to lower the cost of genotyping new markers with a minimal loss of mapping precision. The selection of this subset of plants is based on the number and position of recombinational crossover sites (or breakpoints) detected with the framework marker data in each plant. The breakpoints identified by the ensemble of the selected plants define a set of bins, i.e., chromosome fragments bounded by two adjacent breakpoints or by a distal breakpoint and the telomere, characteristic of each subset. For a given marker, the joint genotype of the selected subset of plants ideally identifies a unique small bin in the genome. The optimal subset of a given size would have the maximum possible number of breakpoints evenly spaced throughout the genome, resulting in a high number of small bins of uniform size. Vision et al. (2000) developed methods and designed a software program (MapPop) to facilitate the selection of optimal (or nearly optimal) subsets from mapping populations.

Diversity analysis

Among pulse crops, Asiatic grams are assigned to subgenus *Ceratotropis* (Piper.) Verdc. and constitute an economically important group of cultivated and wild species for which rich diversity found in India. *Vigna* of the family Fabaceae includes many important pulse-yielding taxa. The genus *Vigna* was originally published by Savi in 1824, which named it after Domenico Vigna, Professor of Botany in Pisa (Baudoin and Maréchal, 1988Out of seven (*V. radiata* (mungbean), *V. mungo* (urdbean), *V. angularis* (adzuki bean), *V. umbellata* (rice bean), *V. aconitifolia* (moth bean), *V. trilobata* and *V. trinervia*) domesticated or semidomesticated forms, *V. radiata* and *V. mungo* are of Indian origin and economically very important. These two species are morphologically very similar and only breeding test show that these two grams have almost completely separate gene pool. For a long time, *V.*

sublobata was considered as common ancestor of both cultigens, until Arora et al., (1973) reported that it includes two distinct wild forms which are not freely cross compatible with each other but could be crossed easily with cultigens, one with *V. radiata* and another with *V. mungo*. Lukoki et al., 1980 reviewed the status of wild *Vigna* population and suggested that two distinct types should be treated as botanical varieties within the respective cultivated species namely, *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris*. However, Babu et al. (1985) did not recognize such varietal status for these wild forms and described them as one species, viz. *V. sublobata* chiefly, due to continuous and more or less overlapping morphological variations observed in natural populations in Indian subcontinent. Though the confusion regarding the taxonomic status of mungbean and urdbean has been settled in an undisputed manner, the identity, taxonomy and their relationship with the wild forms remain ambiguous till date. Also, due to the threatened habitats of the wild forms and increasing realization of wild relatives as potential reservoirs of useful genes, importance of genetic diversity analysis of the wild forms cannot be overemphasized.

Black gram (*Vigna mungo* (L.) Hepper is an important crop of the *Vigna* group. It is also known as urdbean, urad dal, urd bean, urd, urid, black matpe bean, black gram, black lentil. It has a strategic position in Southeast Asian countries for nutritional security and sustainable crop production (Ali and Kumar, 2006).

Characterization and analysis of genetic diversity are central to effective management of plant genetic resources. Characterization provides description of the germplasm while analysis of genetic diversity gives information about the range of genetic variation. Proper characterization helps in unambiguous discrimination between accessions, detecting redundancies and in monitoring genetic change during maintenance. Estimates of genetic diversity are useful to select suitable parents for breeding programmes and to develop in-situ and on-farm conservation strategies (Karihaloo, 2004).

The eukaryotic genome contains repeat regions of different lengths and motifs. The repeat sequences that have less than six base long central motifs are called microsatellites or SSRs.

The term 'microsatellites' was coined by Litt and Lutty in 1989. These loci contain tandem repeats that vary in the number of repeat units between genotypes and are referred to as variable number of tandem repeats (VNTRs) (i.e. a single locus that contains variable number of tandem repeats between individuals) or hypervariable regions (HVRs) (i.e. numerous loci containing tandem repeats within a genome generating high levels of polymorphism between individuals). Microsatellites and minisatellites thus form an ideal marker system creating complex banding patterns by simultaneously detecting multiple DNA loci. Some of the prominent features of these markers are that they are dominant fingerprinting markers and codominant STMS (sequence tagged microsatellites) markers. Many alleles exist in a population, the level of heterozygosity is high and they follow Mendelian inheritance. New alleles with different numbers of repeating units are generated by slippage of the DNA polymerase during DNA replication (Tautz *et al.*, 1986) and unequal crossing over (Levinson and Gutman, 1987). Various functional roles have been attributed to SSRs: for example, hotspots of recombination, regulation and expression of gene and sex determination in various eukaryotes.

Microsatellite sequences converted into PCR-based markers

The simple repetitive DNA sequences of SSRs, which are abundantly spread throughout the genome of eukaryotes {one SSR per 64.6 kb in monocotyledonous and one per 21.2 kb in dicotyledonous species (Wang *et al.*, 1994)}, provide the basis of PCR based co-dominant genetic marker system called Sequence Tagged Microsatellite Sites (STMS). The regions flanking the microsatellites are generally conserved among the genotypes of the same species. Polymerase Chain Reaction (PCR) primers from the regions flanking the repeat regions of genome are used to amplify the SSR containing DNA fragments. The length polymorphism is created when PCR products from the different individuals vary in length as a result of variation in the number of repeat units in the SSR (Powell *et al.*, 1996). This method includes DNA polymorphism using specific primers designed from the sequence data of a specific locus. Primers complementary to the flanking regions of the simple sequence repeat loci yield highly polymorphic amplification products. Polymorphisms appear because of variation in the number of tandem repeats (VNTR loci) in a given repeat motif. Tri- and

tetra nucleotide microsatellites are more popular for STMS analysis because they present a clear banding pattern after PCR and gel electrophoresis. However, dinucleotides are generally abundant in genomes and have been used as markers e.g. (CA)*n*(AG)*n* and (AT)*n*. The di- and tetranucleotide repeats are present mostly in the non-coding regions of the genome, while 57% of trinucleotide repeats are shown to reside in or around the genes. A very good relationship between the number of alleles detected and the total number of simple repeats within the targeted microsatellite DNA has been observed. Thus larger the repeat number in the microsatellite DNA, greater is the number of alleles detected in a large population.

Microsatellite markers are a preferred type of DNA marker used for germplasm analysis and varietal identification, marker assisted selection and genome mapping because they are locus specific, widely dispersed throughout the genome, highly polymorphic due to variation in the repeat units and highly informative because of co-dominant nature (Weber, 1994). Microsatellites are reported to be more variable than RFLPs or RAPDs and have been widely utilized in plant genomic studies. The major drawback of microsatellites is that they need to be isolated *de novo* from species that are being examined for the first time.

Specific markers like STMS (sequence-tagged microsatellite markers) ALPs (Amplicon length polymorphisms) or STS markers have proved to be extremely valuable in the analysis of gene pool variation of crops during the process of cultivar development, and classification of germplasm (Yang *et al.*, 1994). These markers are extremely sensitive and can detect allelic variability during cultivar development (Yang *et al.*, 1994). STS markers specific to chloroplast or mitochondrial DNA have been useful in providing seed and pollen specific markers which can be utilized for the detection of length variation at multiple physically linked sites and may be used to provide haplotype data and thus genotypically unique individual plants (Wu *et al.*, 1998). Also a comparison of patterns of variability detected with biparentally (nuclear) and uniparentally (organellar) transmitted markers can provide complementary information to population and evolutionary biologists. Excellent examples of this are the Poly A mononucleotide repeats in maize (Powell *et al.*, 1995), Poly (TA/AT)

dinucleotide repeats found in liverworts, maize, pea and nonphotosynthetic green plant *Epifagus virginiana*, and a total of 500 chloroplast SSRs identified with repeat motifs greater than 10 repeat units in rice, tobacco, black pine, liverwort and maize (Powell *et al.*, 1996). Though all these marker types provide valuable information regarding the evolution and phylogeny of various species being studied in any given set of samples, the trend is now shifting towards the use of ESTs (expressed sequence tags) for such analysis. This may be so, because in such studies, one actually looks at the evolution of functional genes (Mason-Gamer *et al.*1998 and Deshpande *et al.*1998). With these views in mind the present treatise included the following precise objectives.

Objectives:

i) Elucidation of genetics of resistance to YMV diseases in urdbean

ii) Molecular mapping of genes controlling resistance to YMV.

iii) Studying genetic relationships among important *Vigna* cultivars and wild species using molecular markers.

iv) Study of genetic variability in different gene pools based on molecular gene sequence.

REVIEW OF LITERATURE

The genus *Vigna* is an important legume taxon, widely distributed in tropical and subtropical regions of both hemispheres. It contains about 150 species, divided into seven subgenera. Of these, the subgenus *Ceratotropis,* mainly comprising species found in Asia, has been differentiated into morphologically homogenous group with specialized and complex floral morphology (Maréchal et al, 1980). *Vigna mungo* (L.) Hepper (urd bean) *and V. radiata* (L) Wilczek (mung bean) are the two most important pulse yielding taxa belonging to subgenus *Ceratotropis* of genus *Vigna*. The Indian center is extremely rich and possesses considerable landrace diversity of these species and several allied ancestral wild taxa. *V. radiata* and *V. mungo* are morphologically similar, however minor differences exist in morphology and important plant characters. Variations have also been reported in karyotype, DNA content, proteins, amino acid composition and species hybridity.

2.1 Taxonomy And Species Relationship

The taxonomic and nomenclatural confusion occurred due to incorrect nomenclature by Linneaeus, who named urd bean as *Phaseolus mungo* and mung bean as *P. radiatus*. Verdcourt, 1970 reviewed the genus and on the basis of morphological and biochemical evidences transferred Asian species of *Phaseolus* to genus *Vigna*. Later, *Phaseolus mungo* was named as *V. mungo* and *P. radiatus* as *V. radiata*. Also, it was considered that both these species were evolved from common ancestor *P. sublobatus*, which afterwards named as *V. sublobata*. Also, further misunderstanding occurred due to wrong cytogenitical relationships based on cytology study (De & Krishnan, 1966) and scanning electron microscopy of seed coat pattern (Jain & Mehra, 1980). The latter author contented that *V. sublobata* was not likely the progenitor of mungbean (*V. radiata*) but instead the progenitor of urdbean. Morphological and biochemical studies by Arora et al, 1973 on wild populations of *Vigna* provide the first constructive evidence about evolutionary relationships among *V. radiata-mungo-sublobata-silvestris* complex. They identified two categories, which respectively gave rise to *V. radiata* and *V. mungo*. Lukoki et al, 1980 accepted specific distinction between two forms and their relation as wild ancestor to the cultivated species. They noted that Plx 416 was similar to *V. mungo* biochemically in possessing glutamyl-methionine and its sulphoxide

and morphologically possess narrower stipules, brighter yellow flowers, 6-8 ovules per ovary, erect pods with long white hairs (or glabrescent) and seeds with a raised arillate hilum. Therefore, they recognized these plants as the wild ancestor of black gram. It was described as a new taxon, namely *V. m.* var. *silvestris* Lukoki, Maréchal & Otoul. Lukoki et al (1980), also described Plx 274 similar to *V. radiata* var. *radiata* in possessing a-glutamyl-s-methylcysteine and its sulphoxide in its seeds and have broader stipules, paler yellow flowers, 10-14 ovules per ovary, spreading pods with short brown hairs and seeds with a flat non-arrillate hilum. Chandel (1984) and Miyazaki (1984) also supported the view that mungbean and urdbean were domesticated from *V. radiata* var. *sublobata* and *V. m.* var. *silvestris* respectively on the basis of their biochemical and morphological studies.

2.2 Origin

V. radiata, V. mungo and their proposed wild forms are originated in India. Archaeological records for these species were not found anywhere outside India (Kajale, 1974). Charred grains of mungbean were recorded from Navadatoli (1500-1000 BC), Satavahana Bhokardan (200 BC- 200 AD) and Neolithic Chirand (1800-1300 BC) in Bihar. Carbonized grains of *V. mungo* were discovered from Chalcolithic Navadatoli (1500-1000 BC), Neolithic Piyampalli (1400 BC) in Tamilnadu and Indo Roman Nevasa (50 BC-200 AD) in Maharashtra. Also, charred grains of wild and cultivated species occurring in stratified layers (Kajale, 1977) provide evidences of various stages of evolution from wild to domesticated forms.

Kajale, 1974 pointed out that phytogeographic distribution of ancient grains might not correspond with the present day distribution of wild ancestral forms, or even cultigens. However, Chandel (1984) showed that archaeological evidences correspond very well with the distribution and co-occurrence of present day putative progenitors of mungbean and urdbean. The wild ancestral form of urdbean, *V. mungo* var. *silvestris* corresponded with the archaeological remains at Navadatoli and Bhokardan, while charred grains of mungbean found at Chalcolithic Navadotoli and Neolithic Chirand could be correlated with the occurrence of *V. radiata* var. *sublobata*. Also, there is mention of these species in Vedic text such as Kautilya 'Arthshastra' and in 'Charak Samhita'. These evidences provide strong support for the origin and domestication of mung bean and urd bean in Indian gene center.

2.3 Ecology, Distribution And Domestication

V. radiata and *V. mungo* are cultivated throughout Asia but are characteristically adapted to particular and different agro climatic conditions. *V. radiata* is widely distributed, relatively drought tolerant and well adapted to a range of soil conditions including light soils and limited irrigation. It is cultivated throughout the southern half of Asia, especially India, Bangladesh, Pakistan, Sri Lanka, Thailand, Laos, Cambodia, Vietnam and relatively dry parts of Java, Eastern Malaysia, South China and Central Asia. *V. mungo* on the other hand require wetter conditions and thrive in relatively heavier soils. It is however unsuitable for the wet tropics and grows best, where rainfall is less than 1000 mm per annum. As a crop it is limited to parts of Pakistan, India, Sri Lanka and Burma, but it has recently been taken up in parts of Southeast Asia and elsewhere in the tropics, by Indian immigrants. *V. radiata* var. *sublobata* is the most widely distributed wild species of the subgenus *Ceratotropis*. It grows along the forest margins and grasslands and in disturbed habitats such as roadsides. It is found across equatorial Africa as far west as Benin, also Tanzania, Madagascar; Middle East-Oman; South Asia-India, Bangladesh, Sri Lanka; South East Asia – Myanmar, Thailand, Vietnam, the Philippines, Indonesia; East Asia-China including Taiwan; Papua New Guinea and Australia. *V. mungo var. silvestris* grows in disturbed habitats such as, roadsides. It is found only in India, Myanmar and Thailand.

Domestication is a multistage process, for pulses it includes increase in seed size, reduced pod shattering and importantly loss of germination inhibition (Plitman and Kislev, 1989; Smartt, 1990; Zohary and Hopf, 2000). Archaeology together with the modern day distribution of wild progenitors suggests three distinct centers domestication in India, including South India, Gujarat and the Ganges plain (Fuller 2002, 2006). These domestication events were associated with mid-Holocene, between 4000 and 2000 BC, and occurred amongst local populations of hunter-gatherers in monsoonal India, although some influences from the west due to spread of livestock cannot be ruled out. The *V. radiata* was probably brought under cultivation in the Northwest, probably in the Himalayan foothills of Punjab region, and in the far south, as early finds in both regions are widely separated. Early finds of urdbean come Gujarat and the Northern Peninsula where wild population of these

species persists (V. mungo var. silvestris) without association with wild mungbean (*V. radiata* var. *sublobata*) (Fuller and Harvey, 2006; Tomooka et al, 2003). Early finds in South India of *V. radiata* occur in the driest Savannah environments of Peninsula together with large quantities of Horsegram (*Macrotyloma uniflorum*) and small millets (*Brachiaria ramosa, Setaria verticillata*), and in some cases associated with introduced domesticates (Fuller et al, 2004). This association with other crops, there large quantity and the difference from the habitat of the wild progenitor all imply cultivation of these early mungbeans (Fuller and Korisetter, 2004). However, wild form of mungbean is widely distributed in Africa, Asia and Australia, so domestication more than once cannot be ruled out.

Tomooka et al, (1992) based on seed protein variation in mungbean landraces from Asia, identified regions of protein diversity and proposed two paths of dissemination. Protein type diversity is greatest in West Asia (Afghanistan-Iran-Iraq) rather than India. From this region mungbean was proposed to have moved east by two routes one following a Southern route through India to Southeast Asia. The other pathway followed a northern route along the Silk Road or possibly through India to China.

2.4 Cytology, Crossability And Species Hybridization

Several cytological and cytogenetical studies have been carried out to determine chromosome number (Bose, 1939; Sen and Ghosh, 1961; Coyne, 1964) and chromosome homologies (De and Krishnan, 1966, 1968a, 1968b) in genus *Vigna*. Subsequently series of investigations were made on Cytology, Crossability and Species hybridization (De and Krishnan, 1968b; Dana, 1966a, b, c, d; Biswas and Dana, 1976a, b; Machando et al, 1982). Both mung and urd bean are diploid with chromosome number 2n=2x=22. The investigation related to the organization and evolution of genome of *Vigna* species appeared difficult due to size, large chromosome number and uniformity in shape and size within and between the complements.

Parida et al 1991, studied quantitative DNA variations between and within chromosome complements of *Vigna* species. The different accessions of *V. radiata* did not exhibit significant differences in 2C DNA content. The DNA amount in V. *radiata* narrowly ranged

from 2.63 to 2.70 pg (mean 2.672 pg), whereas, *V. mungo* possessed 2.83 pg. A series of investigations were conducted involving both cultivated and wild progenitors (Rao and Chandel, 1991; Chandel et al, 1991; Sehgal and Chandel, 1991; Audilakshmi and Chandel, 1990). Chromosome number, morphology, nucleolar organization, karyotype symmetry/asymmetry of *V. radiata, V. radiata* var. *sublobata, V. mungo* and *V. mungo* var. *silvestris* were studied. The study revealed significant differences in the karyotypes, both in between and within the cultivated as well as wild progenitor forms. Variations occurred in the number of V, L and J chromosomes. Rao and Chandel, 1991 confirmed in their studies that species/forms of *Vigna* occurring in Indian gene center have evolved directly from their ancestral forms (Chandel, 1984; Chandel et al, 1984) without undergoing any numerical changes in their chromosome complements. The investigation supported the view that basic chromosome number of mungbean and urdbean is x=11. Beside there is overall stability in chromosome morphology and symmetry in cultigens and wild alien species, morphological diversity could not be directly correlated with the karyological differentiation. It was also concluded that *V. radiata var. sublobata* is surely the wild progenitor of mung bean and definitely not of urd bean (*V. mungo*) as reported earlier by Sharma et al, 1980 and Babu et al, 1988.

Crosses performed between mung and urd bean (Dana, 1966a; Sen and Bhowal, 1960) showed presence of sterile hybrids. In these crosses translocations, inversions, deficiencies and duplications in chromosomes were suggested as the possible reasons for hybrid inviability. Similarly, when *V. radiata* var. *sublobata* was crossed with *V. radiata* it produced fertile hybrids, while when crossed with *V. mungo* it resulted in only few shriveled seeds (Karmakar and Dana, 1987). They attributed it to cytogenetic differentiation between *V. radiata* and *V. mungo* that appears to have occurred during process of natural evolution resulting in the speciation of these two very similar taxa. Also, Karmakar and Dana, 1987 reported that wild collection of *V. radiata var. sublobata* (Morphotype 2-Jabalpur) when crossed with *V. radiata* as female parent, they found that *V. radiata* var. *sublobata* was not separated from mung bean by any isolating barriers, while it is separated from urd bean by multiple types of isolating mechanisms.

Comprehensive interspecific hybridization studies were undertaken between *V. radiata* and its allied wild prototype species *V. radiata* var. *sublobata, V. mungo* and its wild ancestral form *V. mungo* var. *silvestris* (Audilakshmi and Chandel, 1990). Different species/accessions were involved in straight as well as in different combinations including reciprocal crosses. The close relationship between wild progenitor species and their cultigens was clearly established with the production of successful hybrids and normal segregations in subsequent generations. However, *V. radiata* var. *sublobata* exhibited only partial compatibility with *V. mungo*, suggesting distinctness. These results were confirmed through meiotic studies as well.

2.5 Biochemical Analysis

The electrophoresis of seed proteins showed marked differences in mungbean and urdbean employing gel electrophoresis technique (Mihardja et al, 1974). Lukoki et al 1980, showed presence of glutamyl 1-s-methylcysteine in mungbean and its wild progenitor *V. radiata* var. *sublobata* while its absence was shown in *V. mungo* and its putative wild progenitor *V. mungo* var. *silvestris*. They also showed absence of glutamyl methionine and its sulfoxide in *V. radiata* and *V. radiata* var. *sublobata* complex, while above-mentioned amino acids were present in *V. mungo* and *V. mungo* var. *silvestris*. Chandel (1980) carried out comprehensive biosystematics studies involving nine cultivated and wild species of *Vigna*. The author carried out comparative morphology, scanning electron microscopy of seed coat cellular structure and hilum structure, chromatography of leaf phenolics and gel electrophoresis of seed proteins. The chromatographic studies revealed a total of 62 phenolic spots in all nine species. *V. radiata* exhibited presence of 17 spots while *V. mungo* possessed 21 spots. V. radiata var. *sublobata* exhibited similar phenolic compounds and their position, and Rf value corresponds with mungbean. Also, wild ancestral form *V. mungo* var. *silvestris* showed remarkable similarity with its putative domesticated form *V. mungo*.

Babu et al, 1988 assessed the seed protein and amino acid composition of *V. radiata* var. *sublobat*a and two cultivated forms *V. mungo* and *V. radiata*. They reported high range of variation for all the amino acids indicating broad genetic base. They suggested the

usefulness of wild populations of *V. radiata* var. *sublobata* in the nutritional upgrading of mung and urd bean. The amino acid profiles were population specific and wild population of *V. radiata* var. *sublobata* were shown to differ contrastly in cysteine content with *V. radiata* as well as *V. mungo*. *V. mungo* contained high methionine content, which could be due to Y-glutamylmethonine and its sulphoxide while *V. radiata* possessed cysteine and its sulphoxide. These results were similar to that reported by Lukoki et al, 1980 and Otoul et al, 1975. However, Babu et al continued to stress upon that *V. mungo* and *V. radiata* var. *sublobata* are closely related. It may be due to the fact that the population of *V. sublobata* they have analyzed could be that of V. mungo var. silvestris as it was collected from Western Ghats and therefore resembled more to *V. mungo*.

Some indirect evidences from nodulation studies also indicate the origin of *V. mungo* and *V. radiata* from *V. mungo* var. *silvestris* and *V. radiata* var. *sublobata* respectively (Kavimandan and Chandel, 1988). Several strains of root nodule bacteria were isolated form *V. mungo, V. radiata, V. radiata* var. *sublobata* and *V. mungo* var. *silvestris*. Wild progenitor *of V. radiata and V. mungo; V. radiata var. sublobata and V. mungo var. silvestris* respectively exhibited distinct differences for bacterial specificity resulting in differential nodulation and the nitrogen accumulation.

2.6 Genomic Characters, Photosynthetic Activity, Seed Protein Content And Amino Acid Levels

Genomic characters such as the variations in the 2C DNA content are related to duration of cell cycle, minimum generation time, latitudinal distribution of agricultural crops and nuclear instability of hybrids (Benett, 1976, 1977, 1982; Smith and Benett, 1975; Rees et al, 1982). The 2C DNA amount was low in wild forms in comparisons to cultivars (Ignacimuthu and Babu, 1988b; Lakhanpaul, 1989). This suggests that evolution of 2C DNA in genus *Vigna* is towards an increase in 2C DNA content. This increase is probably associated with domestication and selection. The wild forms have to adapt to fluctuations in environmental conditions; therefore the lower 2C DNA content enable the plant populations to have shorter mitotic and meiotic cycles. On the other hand, cultivars have been evolved as a result of

directional selection under stable environment; as such the longer duration of mitotic and meiotic cycles may not be a major constraint in the reproductive efficiency of the agricultural populations (Nagato et al, 1981; Resslar et al, 1981). Also, induced chromosomal aberrations in wild relatives are low in comparison to cultivars (Kallo, 1972; Wolff, 1981; Gupta and Roy, 1985; Ignacimuthu and Babu, 1989). This implies that the genomes of the cultivars are rapidly differentiated because of larger number of macro mutations leading to higher rates of sequence turnover (Flavell, 1982; Rees et al, 1982; Rees, 1984). Domestication and selection might have further aided in the rapid differentiation of genomes between cultigens and divergence of the cultigens from wild relatives.

Experiments on photosynthetic activity by Ignacimuthu and Babu (1987, 1989), showed that wild relatives have high photosynthetic rate and RuBPCase activity at vegetative stage as compared to the cultivated forms. This might be due to the high rate of photosynthesis per unit leaf area in wild forms in comparison to the cultigens (Evans, 1983). The cultivars have shown higher photosynthetic activity the reproductive stages due to their larger sink size than the wild relatives (Lorimer, 1981; Mc Cree, 1983). The higher photosynthetic rate at the vegetative stage in wild forms was due to smaller leaf area and higher dry weight of leaf (Ignacimuthu, 1985). The increase in leaf area coupled with the increase in size and number of harvestable organs during the process of selection and domestication led to increase the photosynthetic rate of cultigens, but the Carbon dioxide Exchange Rate (CER) per unit leaf area is low because of larger leaf size (Gifford and Evans, 1981; Yamauchi and Yoshida, 1985). The wild species have productivity promoting gene assemblies and hence of immense value in the development of high yielding varieties (Ignacimuthu and Babu, 1987).

Studies on seed proteins revealed that wild forms are as good as cultivars, nutritionally (Ignacimuthu and Babu, 1984, 1987; Babu et al, 1988). Also the amino acids levels of the wild forms are higher than the FAO standards and are even higher than the cultivated forms (Ignacimuthu and Babu, 1987, 1989; Babu et al, 1988). This genetic variability found in essential amino acids of seed proteins among different population could be used in cross breeding programmes for the nutritional upgrading of modern cultivars

(Gepts and Bliss, 1984). Therefore, the wild populations are nutritionally as good as or even better than the cultigens grown for the human consumption or livestock feed.

2.7 Wild Relatives As Genetic Resources

Fuzi and Miyazaki (1987) reported an accession (TC1966) of *V. radiata* var. *sublobata* that showed perfect resistance against azukibean weevil (*Callasabruchus chinensis*). The resistance was found to control by a single dominant (Kitamura et al, 1988). Fuzi et al (1989) further found that TC1966 is completely resistant against *C. maculates, C. phaseoli* and *Zabrotes subfasciatus*. Tomooka et al (1992) developed a bruchid resistant mungbean line in Thailand by using TC1966 as a gene source. In addition to bruchid resistance, high resistance to yellow mosaic virus (Singh and Ahuja, 1977), high methionine content in seeds (Babu et al, 1988), higher photosynthetic activity and tolerance to drought ((Ignacimuthu and Babu, 1987), higher tolerance to saline and alkaline soils (Lawn et al, 1988) have been reported for *V. radiata* var. *sublobata*. In comparison to *V. radiata* var. *sublobata* there are very few studies of evaluation of *V. mungo* var. *silvestris* as genetic resource. However, this variety is cross compatible with *V. mungo* (Miyazaki et al, 1984). Therefore, further collection and evaluation *V. mungo* var. silvestris is necessary for genetic upgradation of *V. mungo.*

The results of above mentioned studies conducted by several independent workers and support from taxonomic, cytological/cytogenetical and species hybridization studies as well as evidences from biochemical assays, SEM studies and archeological sources indicate that V. radiata and V. mungo are two distinct species. *V. radiata* and *V. mungo* have evolved directly from their proposed wild forms *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* respectively in Indian gene center.

2.8 DNA Based Molecular Markers In Plants

With the advent of DNA based molecular markers over last two decades, the entire scenario of biological sciences has been revolutionalized. Molecular markers have acted as versatile tools and have carved their own niche in various fields like taxonomy, physiology, genetic

engineering etc. They are no longer merely looked upon as DNA fingerprinting markers in variability studies or as forensic tools. Ever since their development, they are constantly being modified, to enhance their utility and to bring about automation in the process of genome analysis. The discovery of polymerase chain reaction (PCR) was landmark in this effort and proved to be a unique process that opened up a multitude of new possibilities in the field of molecular biology. This has facilitated the development of marker based gene tags, map-based cloning of agronomically important genes (Peng et al, 1999), variability studies (Fahima et al, 2002; Thiang et al, 2002) and phylogenetic analysis (Matsuoka et al, 2002a); thus giving new dimensions to concerted efforts of breeding and marker-aided selection for development of newer and better varieties in short time span. These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objectively. Thus it can be discerned that DNA based markers has brought in the much needed amalgamation of classical breeding and modern biotechnological approaches.

Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes. Although DNA sequencing is a straightforward approach for identifying variations at a locus, but until entire genomes are sequenced, geneticists will continue to rely on genetic markers as the means of characterizing the genotypic variation of individuals. A wide variety of techniques have, therefore, been developed in the past few years for visualizing DNA sequence polymorphism and are generally classified as hybridization-based markers and PCR-based markers. In the former, DNA profiles are visualized by hybridizing the restriction enzyme digested DNA to a labeled probe, which is a DNA fragment of known origin or sequence (e.g. RFLP). PCR-based markers involve *in vitro* amplification of particular DNA sequence or loci, with the help of specifically or arbitrarily chosen oligonucleotide sequences 9primers) and a thermostable DNA polymerase enzyme. The amplified fragments are separated electrophoretically and banding patterns are detected by different methods such as staining and autoradiography.

2.8.1 Random Amplified Polymorphic DNA

Random Amplified Polymorphic DNA (RAPD) involves the use of single arbitrary primer in a PCR reaction, resulting in amplification of many discrete DNA products revealing nucleotide sequence polymorphisms. The technique was developed independently by two different laboratories (Williams et. al., 1990; Welsh and McClelland, 1990) and called as RAPD and AP-PCR (Arbitrary primed PCR) respectively. In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA template. If these priming sites are within an amplifiable distance of each other, a discrete DNA product is produced through thermocylic amplification. The polymorphisms between individuals result from sequence differences in one or both of the primer binding sites, and are visible as the presence or absence of a particular RAPD band. Such polymorphisms thus behave as dominant genetic markers.

The RAPD reaction mixture comprises of a target DNA sequence that is exponentially amplified with the help of arbitrary primers, a thermostable DNA polymerase, deoxy nucleotide triphosphates, magnesium and reaction buffer. The reaction involves repeated cycles, each consisting of a denaturation, a primer annealing and an elongation step. In the first step the DNA is made single stranded by raising the temperature to 94^0C (denaturation). In the second step, lowering of the temperature to about 32^0 to 45^0 C results in annealing of the primer to their target sequences on the template DNA (annealing step). In the third cycle, temperature is chosen where the activity of the thermostable Taq DNA polymerase is optimal, i.e., usually 72(C. The polymerase now extends the 3' ends of the DNA - primer hybrids towards the other primer - binding site. Since this happens at both primer-annealing sites on both the DNA strands, the target fragment is completely replicated. Repeating these three step cycles 40 to 50 times results in the exponential amplification of the target between the 5' ends of the two primer binding sites. Amplification products are separated by gel electrophoresis and visualized by ethidium bromide staining.

The RAPD technology has provided a quick and efficient screen for DNA sequence based polymorphisms at a very large number of loci. The major advantage is that no DNA sequence information of target genome is required. Sets of short primers (usually 10 mers) suitable for RAPD amplification are available commercially or can easily be synthesized and,

apart from a thermocycler and an agarose gel assembly, no special equipment is required. When compared with RFLP, RAPD requires small amount of DNA and often-crude miniprep procedures yield DNA of sufficient quantity and quality. It does not require radioactivity or special staining procedures to visualize polymorphisms. Automation is possible at all stages from DNA extraction to data collection and analysis. However, one of the inevitable trade-offs with the technique is that amplification is performed under conditions of low stringency. Consequently, some of the products result from weak complexes between primer and template, and this can result in poor reproducibility for some primers and bands. Another problem reported for RAPD analyses is a low incidence of non-inherited bands. While great majority of RAPD bands are known to be inherited as Mendelian markers, care is needed when drawing conclusions based on a small number of band differences. A limitation of RAPD that cannot be overcome is band dominance. This means that heterozygotes are rarely detected and there are usually only two states for a polymorphism: present and absent. Consequently, RAPD and other multilocus profiles provide less genetic data than profiles for single locus codominant markers such as STS.

RAPD markers have been used for the development of genetic maps, for tagging useful traits and in population genetics. One of the first practical uses of RAPD markers was in the creation of high-density genetic maps. For mapping with RAPD markers it is necessary to use backcross or recombinant population, haploid or gametophytic tissue, or alternatively in a F2 population where only RAPD markers amplified from a single parent are mapped. Genetic simulations show that dominant markers linked in coupling are as efficient for mapping as codominant markers on a per gamete basis.

RAPD has been used to identify molecular markers that lie within regions of a genome introgressed during the development of near isogenic lines. In tomato, RAPD markers specific to chromosome 6 were identified by screening *Lycopersicon esculentum* substitution line (Klein - Lankhorst et. al. 1991). RAPD markers linked to the Pto locus in tomato were identified after screening two near isogenic lines (Martin et. al., 1991).

RAPD markers can also be used to screen for markers linked to specific regions of the genome by the bulk segregant analysis method. This method uses two-bulked DNA samples gathered from individuals segregating in a single population. Each bulk is composed

of several individuals that differ for a specific phenotype or genotype, or individuals at either extreme of a segregating population. Random primers amplify loci from each pool and identify polymorphisms linked to the trait of interest.

The area of research that has shown the most growth with respect to the use of RAPD technology is that of population genetics. RAPD markers have been used to create fingerprints for the study of individual identity and taxonomic relationship in both eukaryotic and prokaryotic organisms. RAPD markers are being used effectively to assess the amount of genetic diversity in germplasm collections. Using only 25 different decamer oligonucleotide primers, Kresovich et al. (1992) collected information on 140 different polymorphic characters in a "test array" of individuals representing B. oleracea and B. rapa. They showed the utility of the assay for discriminating between individuals in a germplasm collection and the ability to distinguish between closely related individuals was simply a function of the number of RAPD bands that were observed.

2.8.2 Inter Simple Sequence Repeats

Inter Simple Sequence Repeats (ISSR) technique is a PCR-based multi-locus marker system that employs oligonucleotide primers homologous to SSR sequences such as (GATA)n to amplify mainly the inter-SSR regions. Amplification products are only obtained only if SSRs in opposite orientation are found in a PCR-able distance, with flanking sequences matching the oligonucleotides. ISSR markers are multilocus unmapped dominant markers. The various synonyms used for ISSR techniques are SSR-anchored PCR; anchored SSR-PCR (ASSR-PCR); microsatellite primed PCR (MP-PCR); anchored microsatellite primed PCR (AMP-PCR); single primer amplification reaction (SPAR); randomly amplified microsatellites (RAM) and randomly amplified microsatellites polymorphism (RAMP). The microsatellite repeats used, as primers can be dinucleotide, trinucleotide, tetranucleotide and pentanucleotide. The primer used can either unanchored (Gupta et al, 1994; Meyer et al, 1993; Wu et al, 1994) or more usually anchored at 3′ or 5′ end with 1 to 4 degenerate bases extend into flanking sequences (Zuetkiewicz et al, 1994). When unanchored i.e. only SSRs are used as primers, the primer tends to slip within the repeat units during amplification leading to smears instead of clear bands. Extending the primer (anchoring) with 1 to 4

degenerate nucleotides the 3′ end or 5′ end assures annealing only to the ends of the micro satellite template DNA thus obviating internal priming and smear formation. Secondly, the anchor allows only a subset of microsatellite to serve as priming sites. When 5′ anchored primers are used, the amplified products include the microsatellite sequences and their length variations across a genome and therefore give more number of bands and higher degree of polymorphism. Usually dinucleotide repeats, anchored either at 3′ or 5′-end reveal high polymorphism (Blair et al., 1999; Joshi et al., 2000; Nagaoka and Oghihara, 1997). The primer anchored at 3′-end give clear banding pattern as compared to those anchored at 5′ ends (Tsumura et al., 1996; Blair et al, 1999; Nagaoka and Oghihara, 1997). Since the primer is a SSRs motif the frequency and distribution of the microsatellite repeat motifs in different species also influence generation of bands.

ISSR technique is nearly identical to RAPD technique except that ISSR primer sequences are designed from microsatellite regions. High stringency of amplification in the form of long primers (16-25 bp) and elevated annealing temperature (45-60° C) makes ISSR more reproducible than RAPD markers. ISSRs segregate mostly as dominant markers following simple Mendelian inheritance (Tsumura et al., 1996; Gupta et al., 1994; Wang et al., 1998; Ratnaparkhe et al, 1998). However they have also been shown to segregate as co-dominant markers in some cases thus enabling distinction between homozygotes and heterozygotes (Wu et al., 1994; Akagi et al., 1996; Wang et al, 1998; Shankar and Moore, 2001).

ISSR finds application in genomic fingerprinting (Charters and Wilkinson, 2000; Prevost and Wilkinson, 1999;Wolff et al, 1995), genetic diversity and phylogenetic analysis (Virk et al, 2000; Qian et al, 2001; Yang et al, 1996; Wang et al, 1998), genome mapping (Backer et al, 1995; Kojima et al, 1998; Arcade et al, 2000), gene tagging and marker assisted selection (Akagi et al, 1996; Levin et al, 2000; Hantula et al., 1996), determining SSR motif frequency (Blair et al, 1999; Nagaoka and Oghihara, 1997) and studies on natural population/speciation (Qian et al, 2001; Wolff et al, 1995).

2.8.3 Amplified fragment length polymorphisms

Amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995) are Polymerase Chain Reaction (PCR) based markers for DNA fingerprinting and rapid screening of diversity. It combines reliability of RFLP with the power of PCR. The technique is based on the selective PCR amplification of subsets of restriction fragments from a total digest of genomic DNA. It comprises of the restriction of DNA with two restriction enzymes preferably a hexa cutter and a tetra cutter, the ligation of double stranded (ds) oligonucleotide adapter to the ends of the restriction fragments, the amplification of a subset of the restriction fragment using two primers complementary to the adapter and a restriction site sequences, and extended at their 3' ends by 'selective' nucleotides, gel electrophoresis of the amplified restriction fragments on denaturing polyacrylamide gels (sequence gels) or on capillary based gels and the visualization of the DNA fingerprints by means of autoradiography, phosphoimaging or other methods.

The Amplified fragment length pattern generated by means of the AFLP technology are known as AFLP fingerprint. The AFLP fingerprints are rich source of restriction fragment polymorphisms, termed AFLP markers. The frequency with which AFLP markers are detected depends on the level of sequence polymorphism between the tested DNA samples.

Single nucleotide changes will be detected by AFLP when an actual restriction site is affected, or nucleotides adjacent to the restriction sites are affected, which cause the AFLP primers to mispair at the 3' end thus preventing amplification. The selective nucleotides are not an exact match of the sequence next to the restriction sites. In addition, deletions, insertions and rearrangements affecting the presence or size of restriction fragments will result in polymorphism detected by AFLPs.

The AFLP is the random amplification technique, which in contrast to most other random amplification techniques makes use of stringent PCR conditions. The primers are generally 17-21 nucleotide in length and anneal perfectly to their target sequences i.e. the adapter, restriction site and small number of nucleotides adjacent to the restriction sites. This renders AFLP a very reliable and robust technique, which is unaffected by small variations in

amplification parameters (e.g. thermal cyclers, template concentration, PCR cycle profile). The high marker densities that can be obtained with AFLP are an essential characteristic of the technology: a typical AFLP fingerprint contains between 50 to 100 amplified fragments, of which upto 80% may serve as genetic markers. Moreover, it requires no sequence information or probe collections prior to the generation of AFLP fingerprints. AFLP markers usually exhibit Mendelian inheritance, indicating that they are unique DNA fingerprints.

The AFLP markers are used in genetic studies, such as: Biodiversity studies, in analysis of germplasm collection, in the genotyping of individuals and genetic distance analysis and in the identification of closely linked DNA markers. AFLP markers are also used in the construction of genetic DNA marker maps and in the construction of physical maps using genomic clones such as YACs and BACs. The use of AFLP in precision mapping of genes and the subsequent isolation of genes is also well documented.

The AFLP is relatively fast (samples can be processed on automated thermal cycler and DNA sequencer) and assays the entire genome for polymorphic marker. A relatively small amount of genomic DNA (0.05 to 0.5 µg) depending upon the size of genome is required. AFLPs provide 10 to 100 times more markers and are more sensitive than other fingerprinting techniques. AFLPs are highly reproducible, no prior information of the genome is required and also no other taxon specific primer sets are required. Primers are commercially available that work for most of the crops. AFLPs are reliable and informative multilocus probes (Winfeild et al., 1998), and provides high levels of resolution to allow delineation of complex genetic structures (Powell et al., 1996). A high multiplex ratio (no. of different genetic loci analyzed simultaneously per experiment) is possible since AFLP markers are distributed across the genome.

2.8.4 Microsatellites

Microsatellite are tandem repeats of short (2-5 bp) DNA sequences, the most abundant being dinucleotide repeats and represent a significant portion of higher eukaryotic genome (Litt & Luty, 1989). These types of reiterated sequences have been termed as differently as Simple Sequence Repeats (SSRs, Jacob et al, 1991), Microsatellite (Litt & Luty, 1989) or Short Tandem Repeats (Edward et al, 1991). The number of times the unit is repeated in a given

microsatellite can be highly variable, a characteristic that makes them useful as genetic markers. Majority of microsatellite occur in gene introns or other non-coding regions of the genome; thus variation in the number of repeats has no consequence on gene function. Generally microsatellite itself does not cause any phenotype, but rather is used as marker to identify a specific chromosome or locus. When being used as marker, the specific number of repeats in a given microsatellite is not important, but rather the difference in the number of repeats between the alleles. The variation in number of repeats affects the overall length of microsatellite, a characteristic readily measured by laboratory techniques. This class of genetic polymorphism is commonly used for diversity analysis, mapping, linkage analysis and tracing inheritance pattern.

The DNA sequences flanking microsatellite are generally conserved allowing the selection of PCR primers that will amplify intervening SSRs. Variation in number of tandem repeats results in PCR product variation. The high degree of polymorphisms of SSRs results from high copy number of the basic motifs or internal heterogeneity of the sequences. Microsatellite markers has also been referred to as Simple Sequence Length Polymorphism (SSLP, Tautz 1989) Sequence tagged Microsatellite (STMS, Beckmann & Soller, 1990)

The procedure for STMS primer directed PCR is also very simple. The region containing the microsatellite is amplified is amplified by PCR using primers that flank the microsatellite. The numbers of repeats present in the microsatellite or that allele determines the size of the DNA amplified. If the number of repeats of one allele were different from the other, than two separate bands would show up on the gel.

A wide variety of methods for construction of libraries enriched for microsatellite sequences have been reported including those based on vectorette PCR using anchored primers (Lench et al, 1996), hybridization of adapter ligated DNA to the primer bound filters (Kandpal et al, 1994; Edwards et al, 1996), extension of uracil containing DNA templates using repeat primers (Ostrander et al, 1992) and hybridization of PCR generated single stranded template to biotinylated oligos (Kijas et al, 1994). Most of these involved in construction of either genomic or cDNA libraries in the Plasmid or phage vectors.

Majority of approaches for the preparation of microsatellite markers is based either on the computer analysis of DNA sequence databases or the screening of various kinds of

genomic DNA libraries. In order to improve efficiency, microsatellite-enriched libraries have been constructed using various methods. Over the years the consensual approach has been developed that is largely based on the adapter-ligation method described by Kandpal, 1994 and Hamilton, 1999. This involves shearing or sonication of genomic DNA to 400 bp-1000bp sizes followed by ligation of universal adaptors. Then various di, tri or tetra repeat oligos are end labeled with biotin and hybridized to adaptor ligated size fractionated DNA. The corresponding is then captured using the streptavidin coated magnetic particles and separated on magnetic rack. These captured fragments are then eluted, washed and ligated to vector of choice and transformed to E. coli preferably using blue white selection. The transformants are then picked up on a nylon membrane and screened using same oligos that were earlier used for capture. These positive clones were sequenced subjected to various software's like SSRIIT and TRF for analysis. Subsequently primers are designed flanking the repeat and used for amplification.

Microsatellites are used as DNA markers for a variety of purposes. They can be used to construct genetic maps for e.g. to investigate QTLs for various traits. They may also be used to investigate genetic diversity, both within and between species. Microsatellites are suitable markers to investigate intra-specific gene flow and demographic parameters such as population size fluctuation. They may also be suitable for phylogenetic studies.

STMSs are easier to use than RFLPs owing to the smaller amount of DNA required, higher polymorphism and ability to automate assays. These markers can be easily exchanged between researchers because each locus is defined by primer sequences. STMS assays are more robust than RAPDs are more transferable than AFLPs. They are now replacing RFLPs in genetic mapping of crop plants. A combination of STMSs and AFLPs is used to produce detailed genetic maps.

Unlike PCR with arbitrary primers, sequence-tagged sites (STS) are primers that are based on some degree of sequence knowledge. These unique, sequence-specific primers detect variation in allelic, genomic DNA. STS have a particular advantage over RAPDs in that they are codominant, that is, they can distinguish between homozygotes and heterozygotes. They also tend to be more reproducible, because they use longer primer sequences. However, they have the disadvantage of requiring some pre-existing knowledge

of the DNA sequence of the region, even if only for a small amount. The investment in effort and cost needed to develop the specific primer pairs for each locus is their primary drawback. As with RAPDs, using PCR produces a quick generation of data and requires little DNA. All STS methods use the same basic protocols as RAPDs (DNA extraction and PCR) and require the same equipment.

2.9 Gene flow in relation with *Vigna* spp.

Recent studies by Yamamoto *et al.*, (2005) have indicated that spontaneous interspecies crossing commonly occurs among vascular plants, and therefore that horizontal gene flow from transgenic plants into wild relatives is unavoidable. Few surveys, however, have been conducted to determine an actual flow frequency for individual plant species. The results indicate that gene flow actually occurs among *V. angularis* complex plants, and that transferred genes might be stably maintained by the offspring. The present observation cannot be directly applied to transgenic plants due to the lack of information as to whether or not artificially introduced genes behave as do native genes after horizontal movement. However, as gene flow is found to be inevitable in this species, specific assessment of whether or not the gene to be introduced confers a significant selective advantage to the host is critical for utilization of genetically modified plants in future.

MATERIAL & METHODS

3. MATERIALS AND METHODS

Materials

3.1 Plant materials

To attain the objectives of mapping MYMV resistance with DNA markers, it was proposed to develop F_2 population from a cross between IPU 982, an improved high yielding recommended variety but highly susceptible to MYMV infection (Figure 1), and Cuttack Local, a local landraces (LLRs) from the Cuttack district of Orissa known to be a low yielder but resistant to MYMV disease. The cross was done at Allahabad and the F1 progenies were also planted there. The F2 generation was grown in NBPGR, New Delhi along with the parents.

For diversity analysis a total of 375 individuals of 25 accessions of (ten from each of *vigna mungo, vigna sylvestris and* five from *vigna hainiana*) were used. The sample details are in table 3.1.

Table 3.1: Accessions of diversity analysis.

Pop Id	Species	Acc.
1	Mungo Cv	BBD-08-01B
2	Mungo Cv	BBD-14-01B
3	Mungo Cv	BB-03-09
4	V.Hyniana	BB-07-01A
5	V.Hyniana	BBD-15-01A
6	V. Mungo	BB_03-10
7	v.Silvistris	BBL-63-2K
8	v.Silvistris	BBL-76-2000

9	V.Silvistris	BBL-80-2K
10	V.Mungo	IPU-99/10
11	v.Silvestris	BBL-50-2K
12	V.Silvestris	BBL-40-2K
13	V.Mungo	Vamban 2
14	V.Mungo	PDU-1
15	V.Mungo	TMV-1
16	V.Mungo	PDU-1016
17	V.Mungo	UG-414
18	V.Silvestris	BBL-55-2K
19	V.Hainiana	KP3-Rajgarh
20	V.Silvestris	IC-251-407
21	V.Hyniana	IC-251-377
22	V.Silvestris	BB-2638
23	V.Silvestris	BB-2641
24	V.Silvestris	BB-2642
25	V.Hainiana	BB-2649

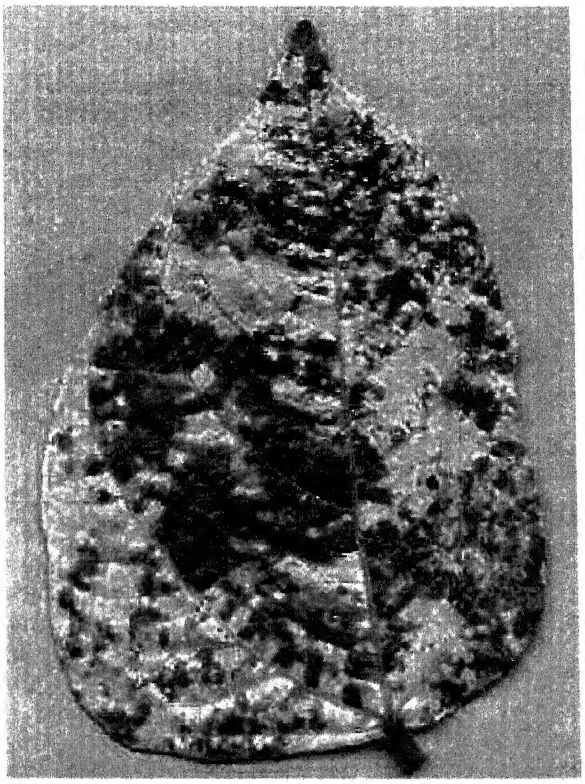


Figure 1: Representation of MYMV affected leaf.

3.2 Molecular marker analysis

3.2.1 DNA isolation

Extraction of total genomic DNA was carried out using CTAB method given by Saghai-Maroof *et al.* (1984) with minor modifications of Porebski *et al.* (1997) and Khanuja *et al.* (1999). Solutions, reagents and instruments are described in Appendix I.

Five gram of young and healthy etiolated seedling material per accession were ground to fine powder in pre-cooled mortar and pestle in liquid nitrogen. Powdered material was quickly transferred to centrifuge tubes containing 15 ml pre-heated CTAB extraction buffer and vortexed to disperse the tissue powder. The tubes were then incubated at 65° C for 1 hour, with intermittent shaking. At the end of the incubation, tubes were cooled to room temperature and 15 ml chloroform: isoamyl alcohol (24:1) was added, following which mixing of contents by inversion was carried out for about 10 minutes. The samples were then centrifuged at 25° C for 15 minutes at 12,000 rpm. The aqueous layer was transferred to fresh centrifuge tubes and the DNA was precipitated by adding 0.7 volume of isopropanol. DNA was spooled out carefully and excess chemical was drained out. DNA was placed in a 1.5 ml microtube, washed two times with 70% ethanol and dried under vacuum. DNA pellet was dissolved in minimum volume of T: E (10:0.1) buffer.

The dissolved DNA was transferred to 2ml Eppendorf tubes; 2µl of RNase A (25 mg/ml) was added and incubated for 1 hour at 37° C. Subsequently, DNA was treated with an equal volume of phenol and spun in a microcentrifuge at 12,000 rpm for 15 minutes at 25° C. This step was repeated with phenol: chloroform (1:1 mixture) and chloroform: isoamyl alcohol (24:1 mixture). RNA-free, purified DNA was precipitated by adding 0.1 volume sodium acetate and 2.5 volumes chilled absolute ethanol to the aqueous phase and then collected by spinning at 12,000 rpm for 15 minutes. The precipitate was washed twice with 70 % ethanol, air-dried and dissolved in TE buffer.

DNA concentration was determined by using DyNA Quant Fluorometer. Prior to quantification, the fluorometer was calibrated with 2 µl (1 mg/ml) of standard calf thymus DNA in 2 ml of low range assay solution (Appendix I). For quantification, 2 µl of DNA sample was added to 2 ml assay solution, mixed thoroughly and measurement in ng/µl recorded.

Part of each DNA sample was diluted with sterilized distilled water to yield a working concentration of 20 ng/µl. The diluted samples were stored at 4°C for immediate use while the original concentrates were kept for long term storage at –20°C.

3.2.2 DNeasy® Plant Miniprep Kit (Qiagen)

To the 100 µl of grounded tissue added 400 µl of lysis buffer (AP1) and 4 µl of RNase solution (100mg/ml) and vortexed vigorously. Mixture was incubated at 65^0 C for 10 min and mixed 2-3 times during incubation by inverting tubes. To the lysate added 130 µl of precipitation buffer and incubated mixture for 5min on ice. Lysate was applied to QIA shredder spin column (lilac) placed in a 2 ml collection tube and centrifuged for 2min at 14000 rpm. Flow through was transferred to a fresh collection tube. To the cleared lysate 1.5 volumes of binding buffer (AP3/E) was added and mixed by pippetting. 650 µl of this solution was applied to DNeasy mini spin column sitting in 2ml collection tube and tubes were centrifuged for 1 min at 8000 rpm. Flow through was discarded. To the DNeasy mini spin column 500 µl of washing buffer (AW) was added and tubes were centrifuged for 1 min at 8000 rpm. Washing step was repeated once again. The DNeasy mini spin column was transferred to a fresh 1.5 ml microfuge tube and 100 µl of elution buffer (AE) applied to the column. Columns were incubated at room temperature for 5 min and centrifuged for 1min at 8000 rpm to elute. The DNA was obtained as flow through. The eluted DNA was stored at -20°C

3.3 Random Amplified Polymorphic DNA (RAPD) analysis

3.3.1 DNA Amplification

Sixty six deca-nucleotide primers from kits A, B, C, D, M and O of Operon technology (USA) was screened for suitability of RAPD amplification. The nucleotide sequences of these primers and characteristics are listed in Table 2. The reaction mixture contained 3mM $MgCl_2$, 0.2 mM dNTPs, 1U Taq (Bangalore genei), 0.4 mM primer and 20 ng genomic DNA. The gene amp 9600 PCR system (Perkin Elmer Cetus, Norwak, CT) was used for amplification with following temperature profile: 94°C for 3 min; 40 cycles of 94°C for 1 min, 32°C for 1 min and 72°C for 1 min; 72°C for 5 min; 4°C end.

Table 3.2: List of screened RAPD primers for mapping population

S.No.	Primer Name	Primer Code	Base sequence (5' to 3')
1	OPA2	S22	TGCCGAGCTG
2	OPA3	S23	AGTCAGCCAC
3	OPA4	S24	AATCGGGCTG
4	OPA5	S25	AGGGGTCTTG
5	OPA6	S26	GGTCCCTGAC
6	OPA7	S27	GAAACGGGTG
7	OPA8	S28	GTGACGTAGG
8	OPA10	S30	GTGATCGCAG
9	OPA11	S31	CAATCGCCGT
10	OPA13	S33	CAGCACCCAC
11	OPA14	S34	TCTGTGCTGG
12	OPA15	S35	TTCCGAACCC
13	OPA16	S36	AGCCAGCGAA
14	OPA17	S37	GACCGCTTGT
15	OPA18	S38	AGGTGACCGT

16	OPA19	S39	CAAACGTCGG
17	OPA20	S40	GTTGCGATCC
18	OPD20	S60	ACCCGGTCAC
19	OPD13	S53	GGGGTGACGA
20	OPB1	S1	GTTTCGCTCC
21	OPB2	S2	TGATCCCTGG
22	OPB3	S3	CATCCCCCTG
23	OPB4	S4	GGACTGGAGT
24	OPB5	S5	TGCGCCCTTC
25	OPB6	S6	TGCTCTGCCC
26	OPB8	S8	GTCCACACGG
27	OPB9	S9	TGGGGGACTC
28	OPB12	S12	CCTTGACGCA
29	OPB15	S15	GGAGGGTGTT
30	OPB16	S16	TTTGCCCGGA
31	OPB17	S17	AGGGAACGAG
32	OPB18	S18	CCACAGCAGT
33	OPB20	S20	GGACCCTTAC
34	OPC2	S62	GTGAGGCGTC
35	OPC4	S64	CCGCATCTAC
36	OPC6	S66	GAACGGACTC
37	OPC10	S70	TGTCTGGGTG
38	OPC13	S73	AAGCCTCGTC
39	OPC15	S75	GACGGATCAG
40	OPC16	S76	CACACTCCAG
41	OPC20	S80	GTTGGGGGAC

43	OPM2	S402	ACAACGCCTC
44	OPM3	S403	GGGGGATGAG
45	OPM4	S404	GGCGGTTGTC
46	OPM5	S405	GGGAACGTGT
47	OPM6	S406	CTGGGCAACT
48	OPM7	S407	CCGTGACTCA
49	OPM8	S408	TCTGTTCCCC
50	OPM9	S409	GTCTTGCGGA
51	OPM11	S411	GTCCACTGTG
52	OPM12	S412	GGGACGTTGG
53	OPM14	S414	AGGGTCGTTC
54	OPM15	S415	GACCTACCAC
55	OPM16	S416	GTAACCAGCC
56	OPM17	S417	TCAGTCCGGG
57	OPM18	S418	CACCATCCGT
58	OPM19	S419	CCTTCAGGCA
59	OPO1	S441	GGCACGTAAG
60	OPO2	S442	ACGTAGCGTC
61	OPO3	S443	CTGTTGCTAC
62	OPO4	S444	AAGTCCGCTC
63	OPO5	S445	CCCAGTCACT
64	OPO6	S446	CCACGGGAAG
65	OPO7	S447	CAGCACTGAC
66	OPO8	S448	CCTCCAGTGT

3.3.2 Preparation of agarose gel

The amplification products were separated by electrophoresis in 1.6% agarose gels according to the procedure outlined below. The gel trays were sealed by casting them in casting trays. The combs were placed in the slots and the whole set up was leveled. A 1.6% mixture of agarose was prepared by dissolving required quantity of agarose (3.84g) in 240ml of 1X TAE buffer. It was boiled in a microwave oven till a transparent mixture was obtained. The mixture was cooled to about 50° C before pouring in gel trays. The trays were allowed to cool till the gel was firmly set. Then, placed in refrigerator at 4° C for 30 mins.

3.3.3 Preparation of samples for loading in PCR tubes

To each 25 ml mixture in PCR tubes, 2.5 ml of bromophenol blue dye (10X) was added and the tubes spun for 5-10 sec.

3.4 Sequence Tag Microsatellites Site (STMS) analysis

For molecular characterization and population diversity analysis, 27 microsatellite primer pairs were used for amplification and resolution of alleles in the 25 *vigna* population.

3.4.1 PCR amplification

3.4.1.1 Optimization of amplification conditions

To determine optimal amplification reaction conditions, PCR was carried out at different concentrations of $MgCl_2$, *Taq* DNA polymerase (0.3 U and 0.5 U per 25 µl reaction mixture) and DNA (10 – 40 mg per 25 ml reaction mixture). The annealing temperatures tested were 3°C above and below the melting temperature, T_m of the particular primer. Conditions were finally optimized as given in Table 3.3, under which clear and consistent bands were amplified. A break-up of the primers based on repeat length and motif is depicted in Fig. 4.

Table 3.3: Primer details for STMS analysis.

Sl. No.	Primer	Primer sequence (5'- 3')	Repeat Unit	Annealing temp. (°C)
1.	AB128093	CCCGATGAACGCTAATGC TG	(AG)	57
		CGCCAAAGGAAACGCAGAAC		
2	MB122A	TGGTTGGTTGGTTCACAAGA	(TGGT)	48
		CACGGGTTCTGTCTCCAATA		
3	AB128113	TCAGCAATCACTCATGTGGG	(AG)	55
		TGGGACAAACCTCATGGTTG		
4.	AB128135	ACTATTTCCAACCTGCTGGG	$(AG)_{46}$	48
		AGGATTGTGGTTGGTGCATG		
5.	MB91	GAGGCCAATCCCATAACTTT	(AG) (GA)	48
		AGCACCACATCAGAGATTCC		
6.	VM22	GCGGGTAGTGTATACAATTTG	(AG)	48
		GTACTGTTCCATGGAAGATCT		
7.	VM24	TCAACAACACCTAGGAGCCAA	(AG)	55
		ATCGTGACCTAGTGCCCACC		
8	VM27	GTCCAAAGCAAATGABGTCAA	(AAT),(TC) (AC)	48
		TGAATGACAATGAGGGTG C		
9.	VM 31	GTGTTCTAGAGGGTGTGATGGTA	$(CT)_{16}$	56
		CGCTCTTCGTTGATGGTTATG		
10	M323b	TGCTTCCTTTTGTCTGAGTTAGAA	(GA)	57
		TGACGGAGAGAGAGAGAGAGAG		

3.4.1.2 Thermal cycler conditions

The PCR amplification was carried out in a DNA thermal cycler (BIOER ^{TM}), which was programmed as follows: Initial denaturation for 4 minutes at 94° C followed by 32 cycles of: 1) Denaturation at 94°C for 30 sec, (2) Primer annealing at optimized T_a of the particular primer (Table 3.3) for 1 minute, (3) Primer extension at 72°C for 5 minutes. A 10 min final extension at 72°C followed at the end of 32 cycles.

3.4.2 Gel electrophoresis

Gels of various concentrations ie. 5%, 6%, 7%, 7.5% and 8% concentration were prepared. For casting, the gel stand was assembled. Gel cassettes were prepared by inserting spacers on two parallel sides of the gel plates. The cassettes were inserted into the casting stand and screws were tightened. Polymerization mixture containing appropriate concentration of acrylamide : bisacrylamide solution, distilled water, ammonium persulphate, TEMED and resolving buffer was poured into the cassette with a syringe. Combs were placed on the open notched side of the gel cassette for forming wells. After polymerization, combs were removed and gels were pre run for 15 min at 100 V.

3.4.3 Data analysis

The amplification products were scored across the lanes comparing their respective molecular weights. Each band was treated as one STMS allele. Scoring of bands was done from photographs. Homology of bands was based on distance of migration in the gel.

3.4.4 Population genetic parameters

Out of the 255 samples, a minimal set of 225 were chosen for population genetics studies. These 225 mango landraces were representative of 6 contiguous geographic regions covering 20 districts of 12 states and union territories of India (Table..). Observed and expected heterozygosities (Ho and He) were used to measure genetic diversity using POPGENE (available online at http://www.ualberta.ca/~fyeh/). Deficits in heterozygosity were measured using fixation indices, reduction in heterozygosity from the Hardy-Weinberg equilibrium

were tested. The test for genome-wide departures from neutrality, such as those produced by demographic processes, the Ewens–Watterson test of neutrality (Watterson 1978, 1986), with probabilities calculated based on both homozygosity and Fisher's exact tests (Ewens–Watterson-Slatkin's exact test; Slatkin 1994, 1996), was performed using the program Arlequin Ver. 2000 (Schneider *et al.* 2000). The Ewens–Watterson test enables the detection of deviations from a neutral-equilibrium model as either a deficit or an excess of homozygosity relative to the number of alleles found at a locus.

3.4.5. Analysis of molecular variance (AMOVA)

The analysis of the distribution of genetic variation across the germplasm groups established a priori was done using the AMOVA option of Arlequin software package (Arlequinsoftware, Schneider *et al.* 2000; available online athttp://lgb.unige.ch/arlequin/). Fixation statistics were produced for individual SSRs and groups of germplasm. The significance of the estimates was obtained through permutation tests, using 1,000 permutations.

3.5 STS Analysis

Six sequence tag microsatellites loci, were used for STS analysis in selected vigna population. Primers were synthesized in order to amplify the target loci. PCR amplification was carried out in 25 µl reactions using 40 ng template DNA, 0.2µm each of the forward and reverse primer, 0.3 Units of *Taq* DNA polymerase and 0.2 mM dNTPs. The PCR amplification was carried out in a DNA thermal cycler (BIOER ^{TM}), which was programmed as follows: Initial denaturation for 15 minutes at 94° C followed by 25 cycles of: 1) Denaturation at 94°C for 30 sec, (2) Primer annealing at optimized T_a of the particular primer for 1 minute, (3) Primer extension at 72°C for 5 minutes. A 10 min final extension at 72°C followed at the end of 25 cycles. Amplified products were initially checked by loading 2 µl PCR product on a 1.5 % agarose gel. The amplified products were purified and sequenced by capillary sequencing. The sequence data was trimmed and edited using Edit with Ridom Trace Edit 1.1.0 Version, Ridom GmBH, Germany. Alignment of sequences in different

cultivars was carried out using ClustalX and finally neighbour-joining trees were generated based on the sequences.

Table 3.4: Forward and reverse primer sequence details for STS

Sl. No	Primer	Primer Sequence (5'- 3')	STS Size (bp)	Annealing Temp.
1	BV165289	ATTCATGATAACTCGTCGGATCGCA	349	55
		TCCAACTACGAGCTTTTTAACTGCA		
2	OLM1	GGTCTGCGAGCTGTTTTTGGAGAAG	342	55
		GCAATTCCCTCCTCAGCTAAAAGTG		
3	UDPGD-B3	TGGTGAAGATTTGCTGCATTGGTGC	202	55
		AAGAAGAGGTTCTTGCCACGGCA		
4	SUSY- 8	TCGCAATGAACCACACAGATTTCA	488	55
		GTCCAACCTTGCCATGGTGAAGATA		
5	SHMT- 1	ACCACAACTCACAAGTCACTTC	570	55
		TTGCTGAGAACCTGCTGTTGGTATG		
6	RNAR- 8	GTTTGGCAGATTGTTGGGGTGAAGA	417	55

3.5.1 Genei Quick PCR purification kit

To the PCR product was added 5 volumes of binding buffer and this mixture was loaded onto a spin column placed in afresh 2.0 ml collection tube. Column was centrifuged at 10000rpm for 1 min and flow through was discarded. Column was placed in a fresh collection tube and to it was applied 500µl of wash buffer I. Column was centrifuged at 10000rpm for 1 min and flow through was discarded. 700µl of wash buffer II was loaded into column and was centrifuged at 10000rpm for 1 min. Flow through was discarded and column in collection tube was spun for 3-5min to remove any traces of buffer. Column was placed in fresh 2.0ml collection tube and to it was added 50µl of the elution buffer. The column was incubated at room temperature for 1 min and then centrifuged for 1min at 10000 rpm to elute. The DNA was obtained as flow through. The eluted DNA was stored at -20°C.

3.5.2 Sequence Tagged Sites (STS) Analysis

For the STS analysis 25 samples from each populations of Vigna species were taken. Ten primer pairs was screened and six gave good amplification. The product was purified either by using gel purification system or by PCR purification kit. The number of segregating sites(S), observed nucleotide diversity per site between any two sequences assuming that the sample is random (p), number of haplotypes (H), haplotype diversity(Hd), average number of pairwise nucleotide differences within population(K), were calculated using DnaSP version 5. The genetic differentiation among the populations was calculated in terms of fixation index (Fst) that estimates diversity within a subpopulation with respect to total genetic diversity. In addition, average number of pairwise nucleotide differences (Kxy), nucleotide substitution per site (Dxy), and net nucleotide substitution per sit (Da) between populations were also calculated. The sequences were edited and the translated amino acids were aligned. The above parameters were also estimated on DnaSP. Phylogenetic analysis was performed by neighbour-joining (NJ) method with kimura 2-parameter distance matrix (Kimura, 1980) MEGA version 3.0 (Kumar et al 2004).

RESULTS

4. RESULTS

4.1 RAPD analysis

4.1.1 Morphological characterization/evaluation

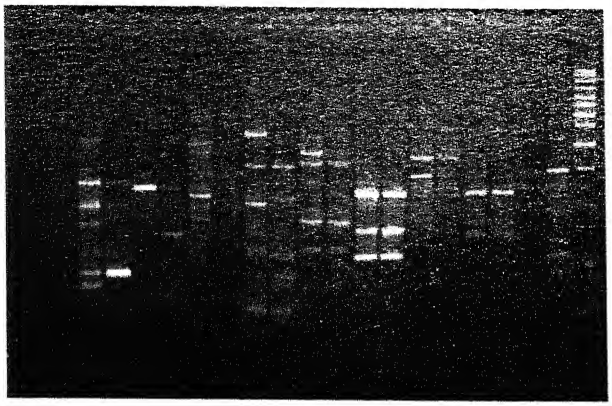
For mapping of MYMV resistance with DNA markers, a F_2 population grown in NBPGR fields, along with the parents from a cross between IPU 982, an improved high yielding recommended variety but highly susceptible to MYMV infection, and Cuttack Local, a local landraces (LLRs) from the Cuttack district of Orissa known to be a low yielding but resistant to MYMV disease. Individuals F_2 plants will be scored following an interaction phenotype (IP) scale of 0-9, where 0 and 1 stands for immunity and resistance, whereas 3, 5, 7 or 9 denote various degrees of susceptibility (Table 4.1).

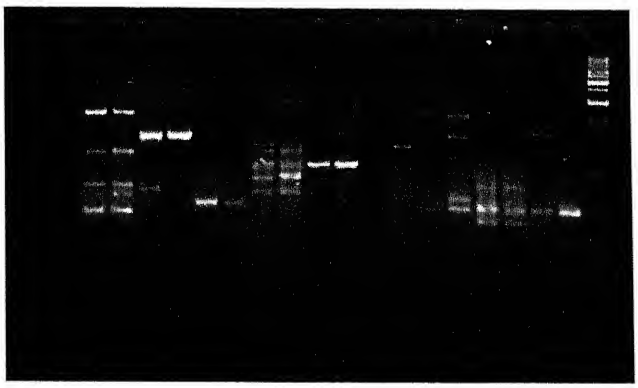
4.1.2 Optimization of RAPD reaction

Template concentration of 20 ng was found to be the ideal concentration as it gave maximum number of reproducible bands. Similar variation in the concentration of Taq DNA polymerase and $MgCl_2$ showed best results with 1U of Taq DNA polymerase and 3mM of $MgCl_2$.

4.1.3 Primer selection and survey

Out of the 66 primers surveyed, 24 were selected for the analyses as others gave either suboptimal, indistinct or monomorphic amplification products (Figure 2).





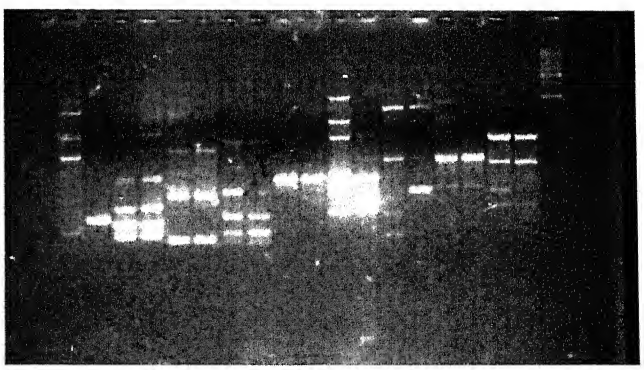


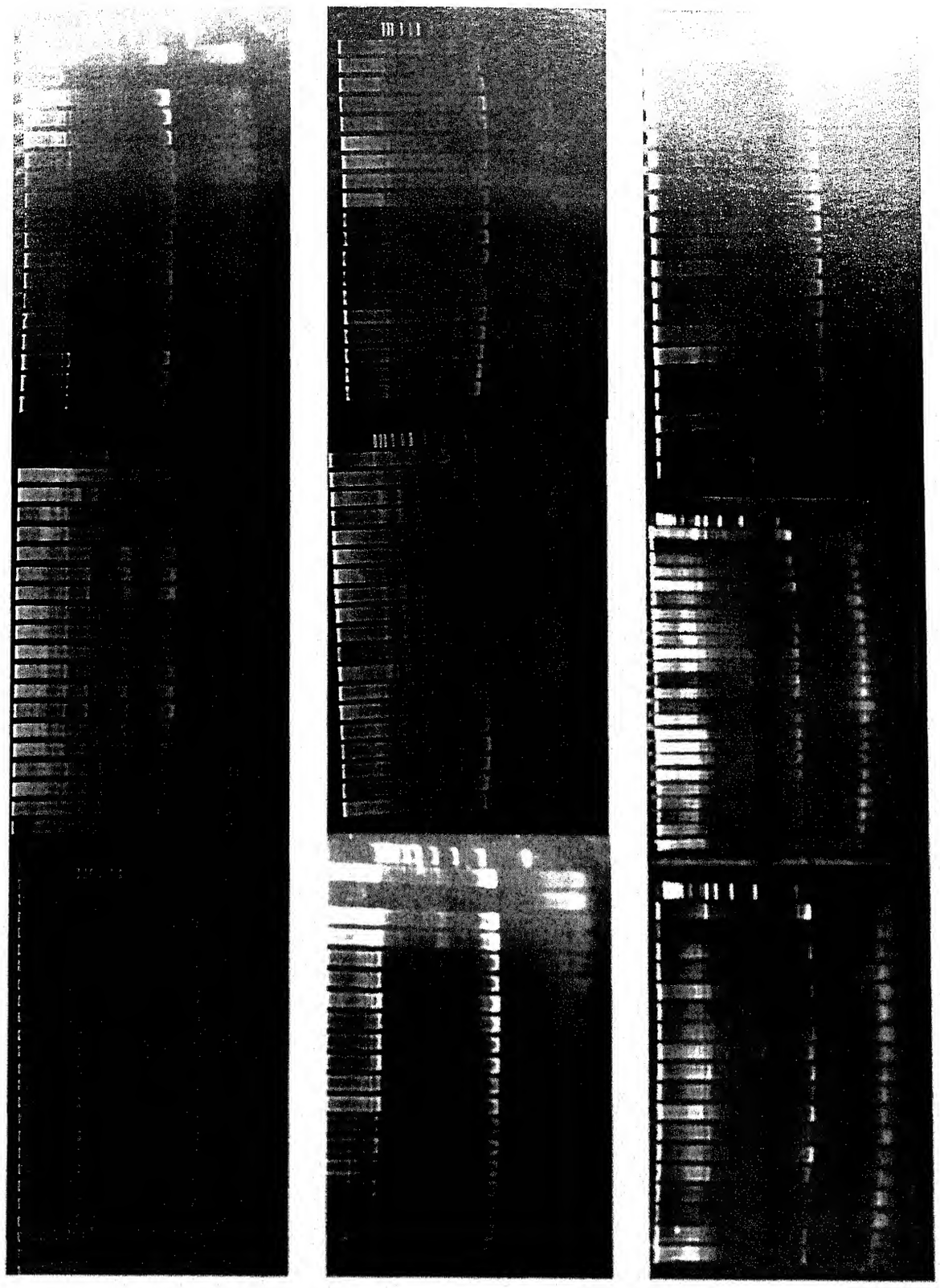
Figure 2: RAPD profiles of parents and F_2 populations, used for screening as indicated in table 3.2.

Table No 4.1 Interaction phenotype score of *Vigna* populations

Sl. No	Plant ID	MYMV	Score
1	CUT LO	B	1.0
2	IPU-982	A	2.0
3	6	A	6.5
4	7	A	7.0
5	11	A	1.5
6	13	A	3.0
7	14	A	5.0
8	15	A	3.0
9	19	A	7.0
10	20	A	5.5
11	22	A	7.0
12	23	A	5.0
13	24	A	8.0
14	29	A	4.0
15	35	A	7.0
16	38	A	3.0
17	39	A	4.0
18	33	A	6.0
19	44	A	5.0
20	46	A	7.5
21	47	A	1.0
22	48	A	5.5
23	53	A	7.5
24	55	A	0.5
25	56	A	2.5
26	57	A	0.5
27	59	A	7.0
28	60	A	5.0
29	64	A	3.0
30	65	A	1.0
31	67	A	5.0
32	68	A	3.5
33	69	A	5.0
34	70	A	7.0
35	75	A	5.0
36	77	A	6.5
37	78	A	4.5
38	79	A	7.0
39	80	A	1.
40	83	A	0.5
41	84	A	3.0
42	86	A	.5
43	87	A	3.5
44	88	A	0.5
45	90	B	
46	92	A	0.5

Legend (Figure 3)

Figure 3: STMS profile of 25 accession of *Vigna* indicating the polymorphism existing for the STMS markers, generated with the primer pairs AB128135. The numbers indicated in each lane correspond to the sequence of accessions as indicated in table 3.1. The lane marked M1 and M2 is the DNA molecular weight standard 100-base pair ladder of MBI. Fermentas, (USA)



4.2 STMS ANALYSIS

4.2.1 Primer screening

A total of 30 STMS primers were screened to identify ideal primers for detailed population genetic studies of 25 populations of *Vigna*. DNA extracted from 15 individual plants per population was used for STMS analysis. The properties of STMS primers, primer sequence, repeat motif, their chromosomal location and annealing temperature are present in Table 2. Of the thirty primers screened, ten polymorphic primers were used in the present study for final STMS analysis. Annealing temperature ranged from 48^0C to 55^0C for the screened primers.

Table 4.2 The number of alleles per locus and their allele size range

Locus	No. of alleles	Allele size (bp)	
		Range	Difference
AB128093	2	180-200	20
MB122A	3	180-250	70
AB128113	4	150-170	20
AB128135	3	150-210	60
MB 91	2	150-180	30
VM 22	3	210-230	20
VM 24	2	150-170	20
VM 27	5	180-230	50
VM 31	2	160-170	10
MB323b	4	300-320	20
Total	30	-	-

Mean	3.0	-	-

4.2.2 Properties of STMS primers

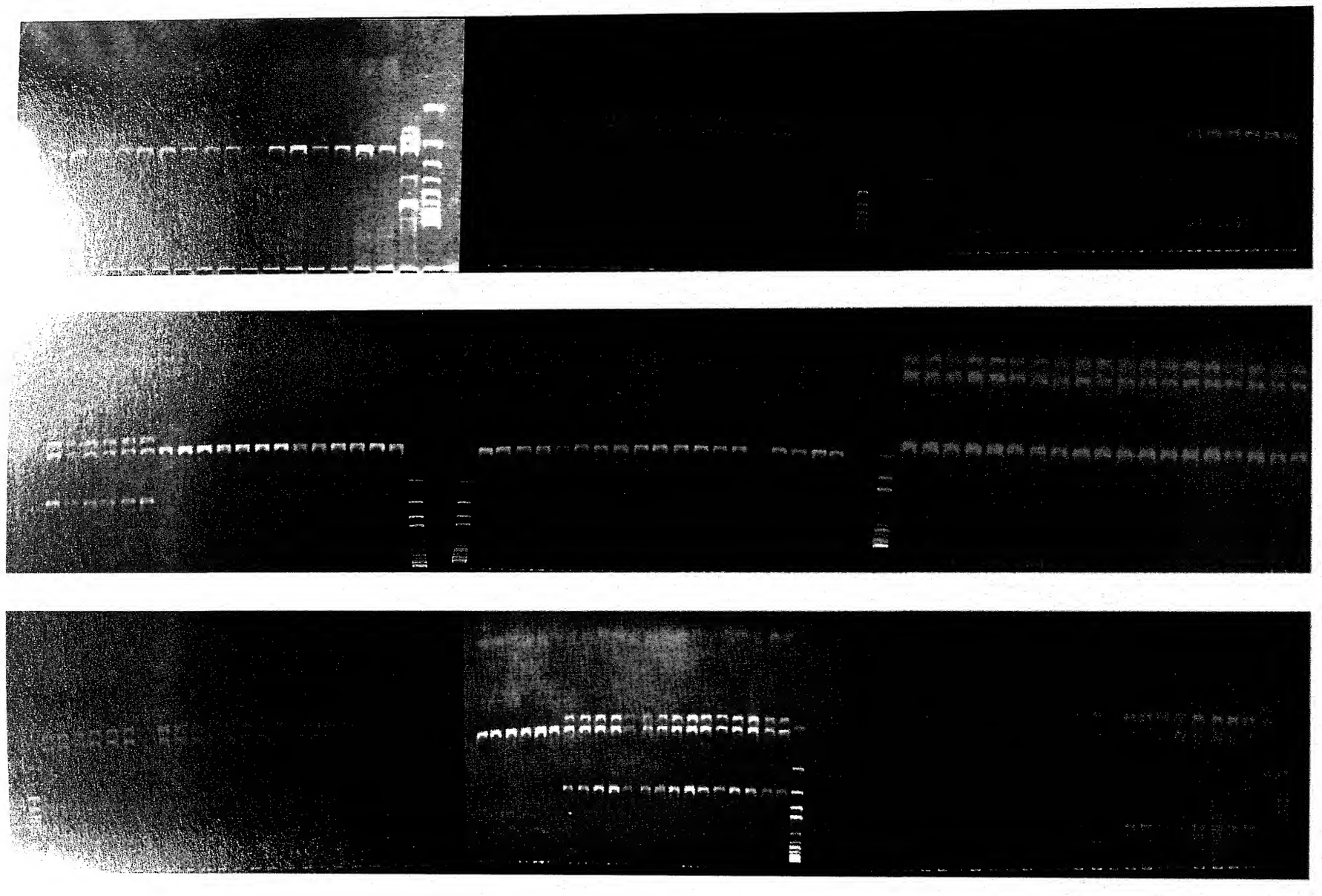
A total of 30 alleles were detected in 25 *Vigna* populations by using ten STMS markers. The number of alleles per locus and their size range are presented in Table 4. The number of alleles per locus ranged from 2 (AB128093, MB 91, VM 24 and VM 31) to 5(VM 27), with an average of 3.0 alleles per locus. The overall size of amplified products ranged from 150 bp to 320 bp. The representative amplification profiles are shown in Figure 3, 4 and 5. The size difference between smallest and largest alleles for a given STMS locus varied from 10 bp (VM 22 and VM 31) to 70 bp (MB122A).

4.2.3 Genetic structure of *Vigna* populations

The genotypes of fifteen individuals of each *Vigna* population were not identical at ten STMS loci which were studied. The allele frequency per locus generated by ten STMS primers in 25 *Vigna* populations was 0.03 to 1.00 presented in Annexure II. The mean number of alleles per locus was 3.0 and that for group I (*Vigna mungo*), II (*Vigna mungo var. silvestris*) and III (*Vigna hainiana*) were 1.70, 2.90 and 2.20, respectively. The number of alleles per locus ranged from 2 (AB128093, VM 22, VM 24 and VM 31) to 5(VM 27). Overall, a total of 30 alleles were detected of which 8 were common (occurring with =0.05 frequency) and 22 were rare (occurring with <0.05 frequency). Of the 25 *Vigna* populations, for all the ten loci, the maximum number of 5 alleles was recorded for the populations BBL-50-2K (*V. silvestris*) and minimum was two alleles for BB-07-01A (*V. hainiana*). The number of alleles per locus ranged from 2 (AB128093, MB 91, VM 24 and VM 31) to 5(VM 27). Population wise the number of observed alleles per locus ranged from 1.0 (*hainiana* BB-07-01A) to 1.9 (*V. sivestris* BB-2642). The genetic diversity in terms of Shannon's information index was highest (0.466) for the population BB-2642 (*sivestris*) and lowest (0.0) for the population BB-07-01A (*hainiana*). The observed heterozygosity was highest (2.0) for the population IC251377 (*hainiana*) and lowest (0.0) for the populations BB-07-01A (*hainiana*). The expected heterozygosity was highest (0.32) for the population BB-2642 (*sivestris*) and a lowest (0.0) for BB-07-01A (hainiana). The overall observed number of alleles and effective

Legend (Figure 5)

Figure 5: STMS profile of 25 accession of *Vigna* indicating the polymorphism existing for the STMS markers, generated with the primer pairs MB 122A. The numbers indicated in each lane correspond to the sequence of accessions as indicated in table 3.1. The lane marked M1 and M2 is the DNA molecular weight standard 100-base pair ladder of MBI. Fermentas, (USA)



number of alleles were 3.0 and 1.7 with the genetic diversity in terms of Shannon's information index was 0.62. The overall observed heterozygosity and expected heterozygosity were 0.08 and 0.35 respectively. The summary diversity analysis of the 25 populations is presented in the Table 4.3.

4.2.4 Genetic structure of grouped populations

Groupwise allele frequency is presented in Table (Annexure II). Groupwise population genetic structure is presented in Table 4.3, 4.4 and 4.5.

Group I (*Vigna mungo*) contain ten populations (BBD-08-01B, BBD-14-01B, BB-03-09, BB_03-10,IPU-99/10,Vamban 2,PDU-1,TMV-1,PDU-1016 and UG-414). The number of alleles detected per locus varied from 1 (AB128113, MB 91, VM 22, VM 24 and VM 31) to 4 (VM27).

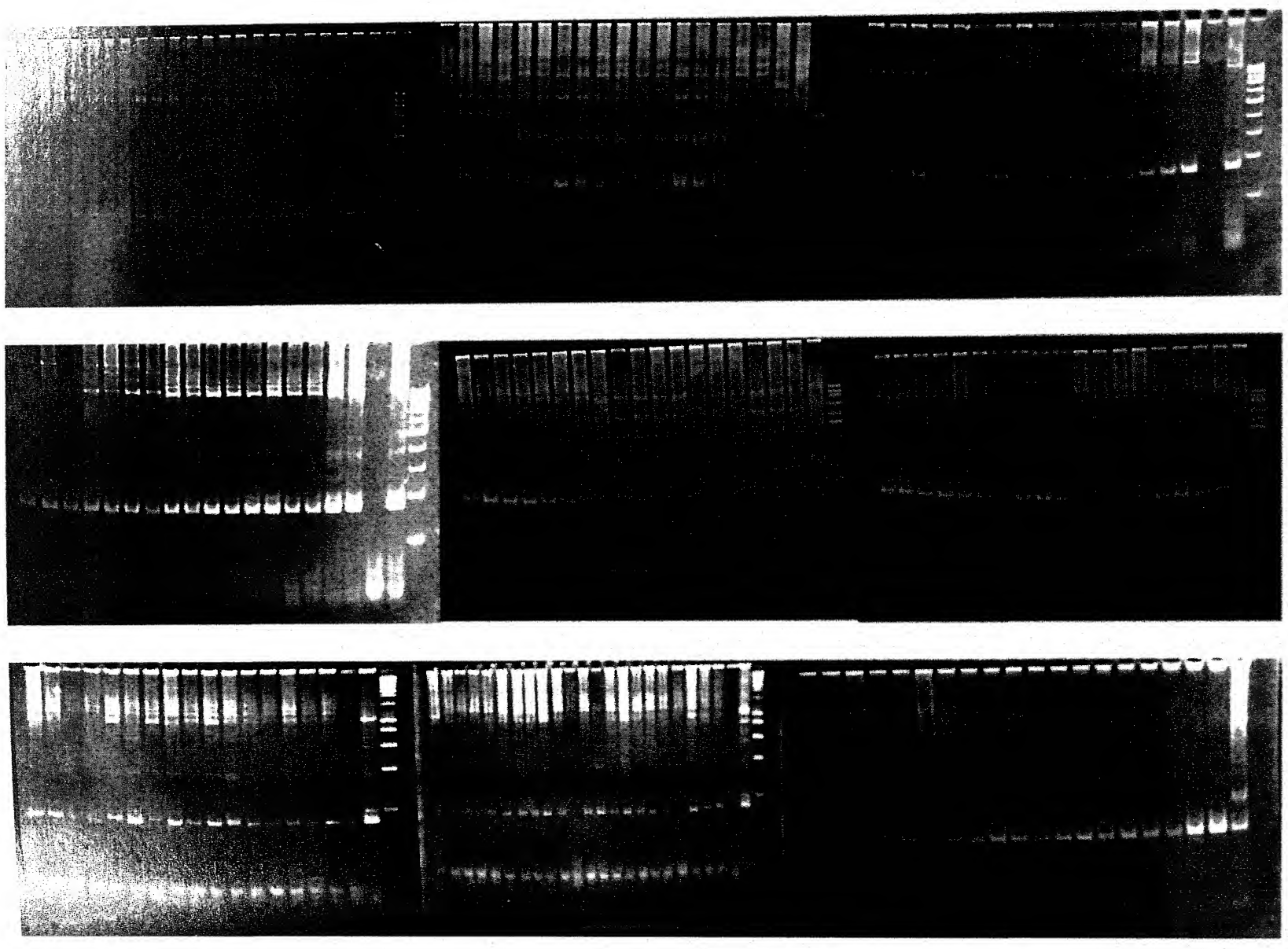
The mean number of observed and effective alleles was detected 1.7 and 1.1. The Shannon's information index was 0.18 for the group 1. The observed heterozygosity and the expected heterozygosity was found 0.083 and 0.10 respectively. The F-statistics and gene flow for the group 1 was found 0.35 and 0.46. The genetic identity and distinctness among the populations of the group is given in the Table 4.6. Dendogram for the group 1 is given Figure 6.

Group II (silvestris) contain ten populations (BBL-63-2K, BBL-76-2000, BBL-80-2K,BBL-50-2K,BBL-40-2K,BBL-55-2K,IC-251-407,BB-2638,BB-2641 and BB-2642). The number of alleles detected per locus varied from 1 (VM 24) to 5 (VM27). The mean number of observed and effective alleles was detected 2.9 and 1.6. The Shannon's information index was 0.57 for the group 2. The observed heterozygosity and the expected heterozygosity was found 0.094 and 0.34 respectively. The F-statistics and gene flow for the group 2 was found 0.51 and 0.24. The genetic identity and distinctness among the populations of the group is given in the Table 4.7. Dendogram for the group 2 is given Figure 7.

Group 3 (*Vigna hainiana*) contain five populations (BB-07-01A, BBD-15-01A,KP3-Rajgarh,IC-251-377 and BB-2649). The number of alleles detected per locus varied from 1

Legend (Figure 4)

Figure 4: STMS profile of 25 accession of *Vigna* indicating the polymorphism existing for the STMS markers, generated with the primer pairs VM27. The numbers indicated in each lane correspond to the sequence of accessions as indicated in table 3.1. The lane marked M1 and M2 is the DNA molecular weight standard 100-base pair ladder of MBI. Fermentas, (USA)



(AB128093, VM 22 and VM 31) to 4 (VM27). The mean number of observed and effective alleles was detected 2.2 and 1.7. The Shannon's information index was 0.52 for the group 3. The observed heterozygosity and the expected heterozygosity was found 0.06 and 0.31 respectively. The F-statistics and gene flow for the group 3 was found 0.76 and 0.07. The genetic identity and distinctness among the populations of the group is given in the Table 4.8. Dendogram for the group 3 is given Figure 8.

Overall in three groups the allele frequency was varied from 0.01 to 1.00 and found high in group 2 than other two groups but observed number off alleles and effective number of alleles was high in group2 (2.9) and group3 (1.7). The observed heterozygosity (0.09) and expected heterozygosity (0.33) was high in group2. The F-statistics and gene flow was high in group3 (0.76) and group1 (0.4647) respectively.

Table 4. 3 Summary diversity of 25 *Vigna* populations based on STMS primers

Pop id	population name	Na	Ne	I	Ho	He	Nei's heterozygosity	Average het
1	mungoBBD8	1.3000±0.4830	1.1284±0.3119	0.1183±0.2262	0.1000± 0.3162	0.0775±0.1635	0.0749 ±0.1581	0.1098 ± 0.1171
2	mungoBBD8b	1.2000±0.4216	1.1565 ±0.3366	0.1253 ±0.2651	0.0533 ±0.1687	0.0901 ±0.1912	0.0871 ±0.1848	0.1098 ± 0.1171
3	mungoBB03	1.1000±0.3162	1.0142 ±0.0449	0.0245 ±0.0775	0.0000± 0.0000	0.0129 ±0.0407	0.0124 ±0.0394	0.1098 ± 0.1171
4	hainBB07	**1.0000±0.0000**	**1.0000 ±0.0000**	**0.0000 ±0.0000**	**0.0000 ±0.0000**	**0.0000 ± 0.0000**	**0.0000± 0.0000**	0.1098 ± 0.1171
5	hainBBD15	1.4000±0.6992	1.2470 ±0.5148	0.1891 ±0.3538	0.0000 ±0.0000	0.1214 ±0.2289	0.1173 ±0.2213	0.1098 ±0.1171
6	mungoBB03_10	1.3000±0.4830	1.1942 ±0.3776	0.1575 ±0.2784	0.1000 ±0.3162	0.1106 ±0.2036	0.1069 ±0.1969	0.1098±0.1171
7	silvBBL63	1.5000±0.7071	1.3535 ±0.6400	0.2524 ±0.3846	0.1000 ±0.3162	0.1657 ± 0.2543	0.1602 ±0.2458	0.1098 ±0.1171
8	silvBBL76	1.5000±0.7071	1.2502 ±0.3832	0.2248 ±0.3170	0.1000 ±0.3162	0.1483 ±0.2147	0.1433 ±0.2075	0.1098 ±0.1171
9	silvBBL80	1.7000±0.8233	1.3519 ±0.4228	0.3134 ±0.3426	0.1000 ±0.3162	0.2053 ±0.2303	0.1984 ±0.2226	0.1098 ±0.1171
10	mungoIPU99	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000 ±0.3162	0.0517 ±0.1636	0.0500 ±0.1581	0.1098 ±0.1171
11	silvBBL50	1.7000 ±0.8233	1.3651 ±0.5275	0.3076 ±0.3739	0.1000 ±0.3162	0.1952 ±0.2417	0.1887 ±0.2337	0.1098 ±0.1171
12	silvBBL40	1.4000±0.5164	1.2072 ±0.3288	0.1979 ±0.2683	0.1000±0.3162	0.1326 ±0.1872	0.1282 ±0.1810	0.1098 ±0.1171
13	mungoVamb2	1.4000±0.6992	1.2133 ±0.4147	0.1799 ±0.3262	0.1000 ±0.3162	0.1161 ±0.2146	0.1122 ±0.2075	0.1098 ±0.1171
14	mungoPDU1	1.4000±0.6992	1.1458 ±0.3175	0.1423 ±0.2524	0.1000 ±0.3162	0.0894 ±0.1718	0.0864 ±0.1661	0.1098 ±0.1171

15	mungoTMV1	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000 ±0.3162	0.0517 ± 0.1636	0.0500 ±0.1581	0.1098 ±0.1171
16	mungoPDU1016	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000 ±0.3162	0.0517 ± 0.1636	0.0500 ±0.1581	0.1098±0.1171
17	mungoUG414	1.2000±0.4216	1.1471 ±0.3342	0.1194 ±0.2557	0.1000 ±0.1811	0.0848 ± 0.1841	0.0820±0.1780	0.1098 ±0.1171
18	silvBBL55	1.3000±0.4830	1.1771 ±0.3330	0.1586 ±0.2653	0.1000 ±0.3162	0.1087 ± 0.1874	0.1051 ±0.1811	0.1098 ±0.1171
19	hainKPSRajg	1.2000±0.4216	1.1923 ±0.4058	0.1366 ±0.2881	0.1000 ±0.3162	0.1014± 0.2138	0.0980 ±0.2067	0.1098 ±0.1171
20	silvIC251407	1.1000±0.3162	1.1000 ±0.3162	0.0693 ±0.2192	0.1000 ±0.3162	0.0517 ± 0.1636	0.0500 ±0.1581	0.1098 ±0.1171
21	hainIC251377	1.2000±0.4216	1.2000 ±0.4216	0.1386 ±0.2923	**0.2000 ±0.4216**	0.1034 ± 0.2181	0.1000 ±0.2108	0.1098 ±0.1171
22	silvBB2638	1.6000±0.6992	1.2691 ±0.3463	0.2623 ±0.3203	0.0667 ±0.1886	0.1687 ± 0.2075	0.1631 ±0.2006	0.1098 ±0.1171
23	silvBB2641	1.6000±0.5164	1.3994 ±0.4567	0.3194 ±0.3209	0.1067 ±0.3146	0.2257 ± 0.2389	0.2182 ±0.2309	0.1098±0.1171
24	silvBB2642	**1.9000±0.7379**	**1.5640 ±0.4306**	**0.4665 ±0.3382**	0.0733 ±0.2319	**0.3175 ± 0.2275**	**0.3069 ±0.2200**	0.1098±0.1171
25	hainBB2649	1.2000±0.6325	1.1273 ±0.4025	0.0892 ±0.2820	0.0000± 0.0000	0.0579 ± 0.1832	0.0560 ±0.1771	0.1098 ±0.1171
--	**Overall**	**3.0000 ± 1.0541**	**1.7386 ± 0.6360**	**0.6174 ±0.3692**	**0.0840 ±0.2500**	**0.3559 ± 0.2190**	**0.3555 ±0.2187**	**0.1098±0.1171**

na = Observed number of alleles, ne = Effective number of alleles [Kimura and Crow (1964)], I = Shannon's Information index [Lewontin (1972)], Ho = Observe heterozygosity, He = Expected heterozygosity, Expected homozygosty and heterozygosity were computed using Levene (1949), ** Nei's (1973) expected heterozygosity.

Table 4.4 Administrative groupwise summary genetic variation statistics for all loci of 25 *Vigna* populations.

Group	Population ID	Na	Ne	I	Ho	He	Nei's heterozygosity	Average het
1	*Vigna mingo*	1.7000 ± 0.9487	1.1904 ± 0.3558	0.1833 ±0.2952	0.0853 ± 0.2698	0.1099 ± 0.1892	0.1095 ± 0.1886	0.1098± 0.1171
2	*Vigna silvestris*	2.9000± 1.1972	1.6435 ± 0.4888	0.5743± 0.3289	0.0947 ± 0.2947	0.3390 ± 0.2053	0.3378± 0.2047	0.1098 ±0.1171
3	*Vigna hainiana*	2.2000± 1.0328	1.7289 ± 0.7692	0.5221 ±0.4454	0.0600 ± 0.1350	0.3186± 0.2744	0.3165 ± 0.2726	0.1098± 0.1171

Table 4.5 F-statistics and gene flow among all the groups.

GROUP	POP	Fis	Fit	Fst	Nm
1	*Vigna mungo*	-0.1985	0.2207	0.3498	0.4647
2	*Vigna silvestris*	0.4305	0.7198	0.5080	0.2422
3	*Vigna hainiana*	0.1921	0.8104	0.7654	0.0766

Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

Table 4.6 Nei's Unbiased Measures of Genetic Identity and Genetic distance (Group 1).

	1	*2*	*3*	*6*	*10*	*13*	*14*	*15*	*16*	**17**
1	****	0.980	0.975	0.952	1.000	0.995	1.000	1.000	0.901	0.937
2	0.020	****	0.977	0.931	0.978	0.983	0.985	0.978	0.957	0.978
3	0.026	0.023	****	0.927	0.974	0.966	0.973	0.974	0.871	0.907
6	0.049	0.071	0.075	****	0.953	0.945	0.952	0.953	**0.852**	0.887
10	**-0.000**	0.022	0.026	0.048	****	0.992	0.999	**1.001**	0.895	0.933
13	0.005	0.017	0.035	0.056	0.008	****	0.997	0.992	0.920	0.951
14	-0.000	0.015	0.027	0.050	0.001	0.003	****	0.999	0.913	0.947
15	-0.000	0.022	0.026	0.048	-0.001	0.008	0.000	****	0.895	0.933
16	0.105	0.044	**0.138**	0.161	0.110	0.084	0.090	0.110	****	0.997
17	0.065	0.022	0.097	0.120	0.070	0.051	0.054	0.070	0.030	****

Figure 6. Dendrogram based on Nei's (1972) Genetic distance: Method = UPGMA
-- Modified from NEIGHBOR procedure of PHYLIP Version 3.5 (Group I)

Table 4.7 Nei's Unbiased Measures of Genetic Identity and Genetic distance (Group II).

	1	*2*	*3*	*6*	*10*	*13*	*14*	*15*	*16*	**17**
1	****	0.980	0.975	0.952	1.000	0.995	1.000	1.000	0.901	0.937
2	0.020	****	0.977	0.931	0.978	0.983	0.985	0.978	0.957	0.978
3	0.026	0.023	****	0.927	0.974	0.966	0.973	0.974	0.871	0.907
6	0.049	0.071	0.075	****	0.953	0.945	0.952	0.953	**0.852**	0.887
10	**-0.000**	0.022	0.026	0.048	****	0.992	0.999	**1.001**	0.895	0.933
13	0.005	0.017	0.035	0.056	0.008	****	0.997	0.992	0.920	0.951
14	-0.000	0.015	0.027	0.050	0.001	0.003	****	0.999	0.913	0.947
15	-0.000	0.022	0.026	0.048	-0.001	0.008	0.000	****	0.895	0.933
16	0.105	0.044	**0.138**	0.161	0.110	0.084	0.090	0.110	****	0.997
17	0.065	0.022	0.097	0.120	0.070	0.051	0.054	0.070	0.030	****

Figure 7 Dendrogram based on Nei's (1972) Genetic distance: Method = UPGMA
■ Modified from NEIGHBOR procedure of PHYLIP Version 3.5 (Group II)

Silv BBL63

Silv BBL80

Silv BBL76

Silv BBL50

Silv BBL40

Silv BBL55

Silv IC251407

Silv BB2638

Silv BB2641

Silv BB2642

Table 4.8 Nei's Unbiased Measures of Genetic Identity and Genetic distance (Group III).

4	*5*	*19*	*21*	*25*
****	**0.9377**	0.7483	**0.4748**	0.6179
0.0643	****	0.7180	0.4984	0.6837
0.2900	0.3313	****	0.7672	0.7561
0.7449	0.6963	0.2649	****	0.5396
0.4815	0.3802	0.2796	0.6169	****

Figure 8 Dendrogram based on Nei's (1972) Genetic distance: Method = UPGMA -- Modified from NEIGHBOR procedure of PHYLIP Version 3.5 (Group III)

Hain BB07

Hain BBD15

Hain BB2649

Hain KPSRajg

Hain IC251377

4.5 Evidence of group differentiation and selection

Group differentiation is indicated by F_{ST} value (Table 4.5), which measures the fixation of alleles in different populations. F_{ST} values ranged from 0.35 to 0.76 this indicates that there is moderate to high genetic differentiation between populations.

Ewens-Watterson test for neutrality was done using 1000 simulated samples (Table 4.9). All the loci were neutral to selection pressure as their observed F value was between lower and upper 95% limit.

Table 4.9 The Overall Ewens-Watterson Test for Neutrality for all loci.

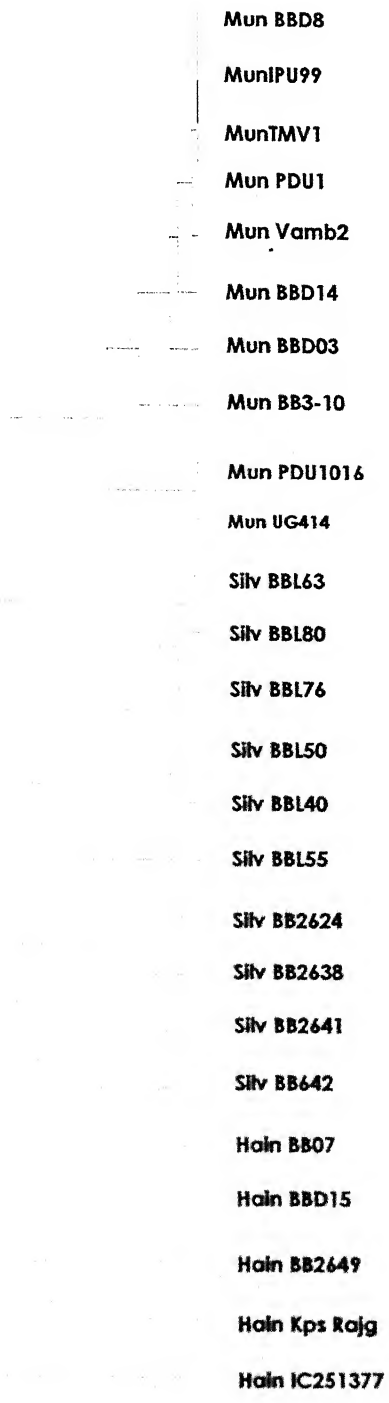
S. No.	Locus	Obs F	L 95	U95
1	AB128093	0.84	0.51	1.00
2	MB122A	0.44	0.39	0.99
3	AB128113	0.54	0.35	0.98
4	AB128135	0.71	0.39	0.99
5	MB91	0.56	0.51	1.00
6	VM22	0.95	0.40	0.99
7	VM24	0.73	0.50	1.00
8	VM27	0.41	0.30	0.97
9	VM31	0.92	0.50	1.00
10	MB323b	0.34	0.34	0.98

Major genetic differentiation was found between wild and cultivated species of *Vigna*. Using POPGENE software, grouped the populations into two broad clusters. The first cluster (Population BBD-08-01B, BBD-14-01B, BB-03-09, BB_03-10, IPU-99/10, Vamban 2, PDU-1, TMV-1, PDU-1016 and UG-414) comprising cultivated varieties of *Vigna* mungo as well as 10 population of (BBL-63-2K, BBL-76-2000, BBL-80-2K, BBL-50-2K, BBL-40-2K, BBL-55-2K, IC-251-407, BB-2638, BB-2641, BB-2642) wild relative *Vigna* sylvestris. With in this cluster the cultivar formed an independent subcluster, closer to subcluster II consist of six populations of *Vigna* sylvestris. Four population of *Vigna* sylvestris (20, 22, 23 and 24) formed subcluster III. Wild population of *Vigna* hainiana (five populations) formed a distant cluster (Figure 9).The genetic identity and distinctness among the populations of the group is given in the Table 4.10.

Table 4.10 Nei's Unbiased Measures of Genetic distance for all the *Vigna* populations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1																									
2	0.21																								
3	0.03	0.02																							
4	0.58	0.58	0.52																						
5	0.38	0.37	0.33	0.07																					
6	0.05	0.07	0.08	0.55	0.38																				
7	0.12	0.15	0.14	0.32	0.30	0.11																			
8	0.13	0.17	0.16	0.37	0.40	0.11	0.03																		
9	0.13	0.14	0.15	0.33	0.30	0.09	0.02	0.02																	
10	0.00	0.02	0.03	0.57	0.37	0.05	0.11	0.13	0.13																
11	0.16	0.18	0.19	0.30	0.24	0.13	0.03	0.09	0.03	0.16															
12	0.22	0.26	0.25	0.28	0.21	0.20	0.03	0.06	0.03	0.22	0.03														
13	0.01	0.02	0.04	0.61	0.40	0.06	0.13	0.15	0.13	0.01	0.17	0.24													
14	0.00	0.02	0.03	0.59	0.38	0.05	0.12	0.14	0.13	0.00	0.16	0.23	0.01												
15	0.00	0.02	0.03	0.57	0.37	0.05	0.11	0.13	0.13	0.00	0.16	0.22	0.01	0.00											
16	0.11	0.05	0.14	0.77	0.52	0.16	0.25	0.27	0.23	0.11	0.27	0.37	0.09	0.09	0.11										
17	0.07	0.02	0.10	0.71	0.47	0.12	0.20	0.22	0.19	0.07	0.23	0.32	0.05	0.06	0.07	0.00									
18	0.21	0.16	0.24	0.55	0.57	0.19	0.14	0.10	0.11	0.22	0.21	0.19	0.20	0.20	0.22	0.12	0.12								
19	1.03	0.99	0.99	0.29	0.33	0.94	0.76	0.73	0.65	1.05	0.63	0.65	0.97	1.05	1.05	1.05	1.03	0.80							
20	0.59	0.48	0.58	0.77	0.77	0.56	0.68	0.66	0.63	0.59	0.69	0.81	0.55	0.55	0.59	0.42	0.43	0.44	0.97						
21	0.65	0.62	0.63	0.75	0.70	0.50	0.66	0.59	0.57	0.67	0.62	0.73	0.60	0.66	0.67	0.67	0.65	0.60	0.27	0.61					
22	0.38	0.28	0.37	0.60	0.57	0.35	0.44	0.43	0.40	0.38	0.46	0.53	0.35	0.36	0.38	0.23	0.24	0.24	0.85	0.08	0.51				
23	0.54	0.44	0.57	0.47	0.61	0.51	0.41	0.39	0.38	0.54	0.44	0.41	0.50	0.50	0.54	0.36	0.37	0.23	0.77	0.22	0.64	0.17			
24	0.31	0.25	0.32	0.48	0.54	0.30	0.31	0.26	0.27	0.32	0.35	0.39	0.27	0.30	0.32	0.23	0.23	0.15	0.60	0.20	0.36	0.11	0.14		
25	0.83	0.80	0.86	0.48	0.38	1.01	0.92	1.05	0.87	0.86	0.77	0.91	0.82	0.85	0.86	0.75	0.76	0.99	0.28	0.87	0.62	0.82	0.89	0.70	

Figure 9 Dendrogram based on Nei's (1972) Genetic distance: Method = UPGMA --Modified from NEIGHBOR procedure of PHYLIP Version 3.5



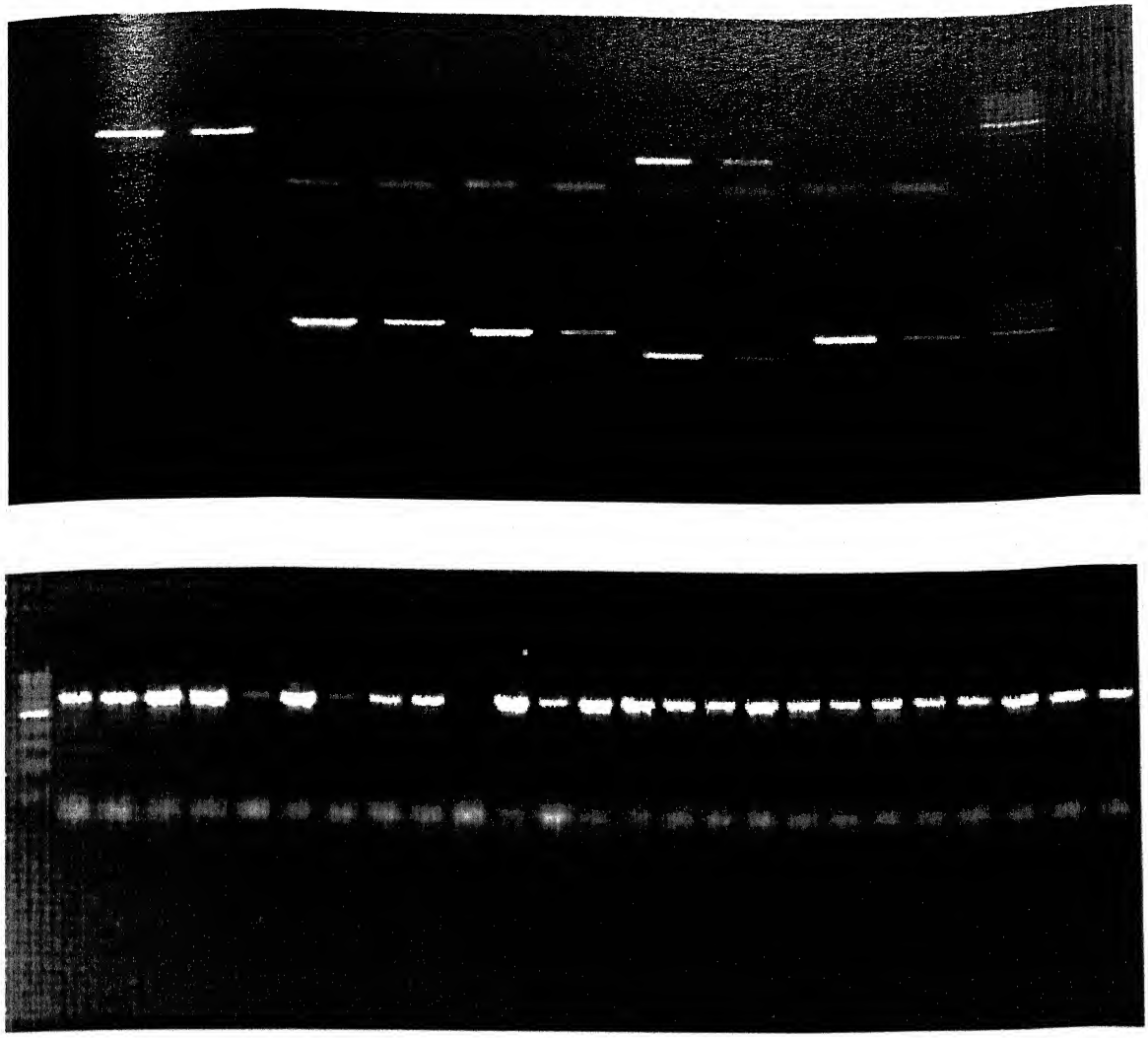


Figure 10: STS profile of 25 accession of *Vigna* indicating the screening of STS markers. The lane marked M1 and M2 is the DNA molecular weight standard 100-base pair ladder of MBI. Fermentas, (USA)

4.6 Analysis of molecular variance

For realizing the partitioning of molecular variance at different levels of hierarchy, Analysis of molecular variance (AMOVA) was performed using Arlequin software. AMOVA revealed maximum percentage of variation within populations (16.03%) followed by 39.13% among populations within group and 44.84% among groups (Table 4.11). Average F-Statistics over all loci were, FSC: 0.710, FST: 0.840 and FCT: -0.840.

Table 4.11 Analysis of molecular variance (AMOVA) at different geographic levels based on Arlequin

Source of variation	Sum of squares	Variance components	Percentage variation
Among groups	333.49	1.25	44.84
Among populations within groups	369.67	1.10	39.13
Within populations	156.40	0.45	16.03
Total	859.56	2.79	

4.7 Sequence Tag Microsatellites Analysis

The present study was undertaken to assess genetic diversity and relationships among selected accessions. Twenty five samples were taken from each population. A total of twelve primers was screened and out of twelve six primers gave reproducible bands and were studied for analysis. Amplified products were purified using PCR product purification kit (Banglore Genei). Purified products were checked by running 2µl of amplified products on 0.8% agarose gel (Figure 10).

4.8 Sequencing

Purified products were sequenced directly using automated DNA sequencer, ABI GA310.

4.9 DNA sequence analysis

The DNA sequence data was first edited using Ridom Trace Edit version 1.1.0 Ridom GmbH, Germany. The clarity of peak separation and height were the criteria used for ascertaining the quality. The acceptable sequence traces were included for further analysis. The sequences were then aligned using CLUSTAL X software (Thompson et al., 1997). The gap penalties and weights were adjusted to obtain reliable alignments. The aligned sequence matrices were used for phylogenetic tree reconstruction using the maximum parsimony, minimum evolution and neighbor joining criteria with MEGA 4.1 software (Kumar et al., 2008). The statistical support for the branches was determined by bootstrap analysis of 5000 psedo-data sets. The percent bootstrap values were indicated on each of the branch point on the trees.

Table 4.12 Measures of DNA sequence polymorphisms and tests of neutrality

Locus	*Segregating site*	*Singleton Variable sites*	*Parsimony Informative site*	*Total no of mutation*	*K*	*H*	*Hd ± S.D*	*p ± S.D*	**Tajima's D**
RNAR8	16	9	7	16	2.58	5	0.967± 0.019	0.01299± 0.00481	-1.639
SHMT 1	3	3	0	3	0.40	3	0.257 ± 0.142	0.00094 ± 0.00058	-1.685
SUSY-8	11	3	4	7	1.24	4	0.333 ± 0.124	0.00907 ± 0.00339	-1.130
BV16	0	0	0	0	0	0	0	0	0
UDPGDB3	5	2	3	5	0.96	3	0.338 ± 0.120	0.00982 ± 0.00365	-0.928
OLM1	47	5	42	53	17.37	14	0.971 ± 0.032	0.07297 ± 0.00478	0.569

S; Number of segregating (polymorphic/variable) sites; **K**; Average number of pairwise nucleotide differences, **H**; Number of haplotypes, **Hd**; Haplotype diversity; **p**; Observed average pairwise nucleotide diversity; **D**; Tajima's D test statistics.

The measures of DNA sequence polymorphism and tests of neutrality was found very high in OLM1 other than five loci. The number of haplotypes in locus OLM1 (H = 14) was higher than RNAR8 (H= 5), SHMT 1 (H= 3) and SUSY 8 (H= 4). The number of segregating site, parsimony informative site and total number of mutations were

highest 47, 42 and 53 in locus OLM1. Singleton variable site found highest 9 in locus RNAR8. The haplotypes (gene) diversity (Hd) was found highest 0.971± 0.032 SD. The Average number of pairwise nucleotide difference (K) was 17.37 with overall **p** diversity 0.07297 ± 0.00478 SD in the locus OLM1 (Table 4.12). The positive value 0.569 for Tajima's D indicates positive natural selection.

Inter population nucleotide difference (Kxy) varied from 0.0235 (between *silvestris* and *mungo*) in locus UDPGDB3 to 26.639 (between *mungo* and *hainiana*) in OLM1. Similarly, the average number of nucleotide (Dxy) and net nucleotide (Da) substitutions per site between populations ranged from 0.00088 (SHMT1) to 0.09146 (OLM1) respectively. Inter-population comparison of the total (TM) versus shared (SM) number of mutation showed only on locus OLM1 (29 of 53 mutations were shared) (Table 4.13). Gene flow was varied from 0.31 (SUSY8) to 8.83 (OLM1) on basis of haplotype data information (Table 4.14). Based on sequence data information it was found 0.15 (SUSY8) to 7.11 (OLM1).

Table 4.13 Inter-population genetic differentiation

Locus	*S. No*	*Population-1*	*Population-2*	*Kxy*	*Dxy*	*Da*	TM/ SM
RNAR8	1	mungo	silvestris	0.717	0.00369	0.00022	4
	2	mungo	hainiana	6.444	0.02740	0.01461	21
	3	silvestris	hainiana	3.359	0.03551	0.01865	14
OLM1	4	mungo	silvestris	17.375	0.07445	0.00309	53/29
	5	mungo	hainiana	26.639	0.09146	0.05232	72
	6	silvestris	hainiana	15.028	0.06015	0.02832	34
SUSY	7	hainiana	mungo	4.045	0.02173	0.01238	16
	8	hainiana	silvestris	2.076	0.02676	0.01622	7
	9	mungo	silvestris	0.444	0.00324	0.00000	4
UDPGB3	10	silvestris	mungo	0.235	0.00255	0.00000	2
	11	silvestris	hainiana	1.561	0.02525	0.01515	5
	12	mungo	hainiana	1.154	0.02273	0.01515	3
SHMT	13	mungo	silvestris	0.400	0.00088	0.00085	3

Kxy; Average number of nucleotide differences between populations

Dxy; The average number of nucleotide substitutions per site between populations,
Da; The number of net nucleotide substitutions per site between populations,
TM, total number of mutations in both populations; **SM**, Numbers of mutations shared between populations

Table 4.14 Gene Flow Estimates based on sequence analysis.(Nei 1973)

	Haplotype Data Information		Sequence Data Information				
Primer	GST	Nm	DeltaSt	GammaSt	Nm	Fst	Nm
SHMT 1	0.03493	6.91	0.00006	0.06250	3.75	0.501	0.25
RNAR8	0.25064	0.75	0.00656	0.53288	0.22	0.00	
OLM1	0.02752	8.83	0.00584	0.08384	2.73	0.034	7.11
SUSY8	0.44439	0.31	0.00514	0.57108	0.19	0.628	0.15
UDPGDB3	0.42677	0.34	0.00546	0.58416	0.18	0.600	0.17

Table 4.15a Population wise Measures of DNA sequence polymorphisms and tests of neutrality for RNAR8.

S. no	*Population*	*S*	*h*	*Hd*	*K*	*Pi*	JC, PiJC
1	mungo	2	2	0.33333	0.66667	0.00335	0.00337
2	silvestris	2	2	0.35556	0.71111	0.00357	0.00360
3	hainian	9	2	0.66667	6.00000	0.03015	0.03110
	Total	16	5	0.53216	2.58480	0.01299	-----

Number of segregating sites, S: Number of haplotypes, h: Haplotype diversity, Hd: Average number of differences, K: Nucleotide diversity, Pi: Nucleotide diversity with JC, PiJC:

Table 4.15b UDPGDB3

S. no	*Population*	*S*	*h*	*Hd*	*K*	*Pi*	JC, PiJC
1	silvestris	2	2	0.25000	0.50000	0.00510	0.00517
2	mungo	0	1	0.00000	0.00000	0.00000	0.00000
3	hainiana	3	2	0.50000	1.50000	0.01531	0.01563
	Total	5	3	0.33810	0.96190	0.00982	------

Table 4.15c SUSY8

S. no	*Population*	*S*	*h*	*Hd*	*K*	*Pi*	JC, PiJC
1	hainiana	3	2	0.66667	2.00000	0.01460	0.01482
2	mungo	0	1	0.00000	0.00000	0.00000	0.00000
3	silvestris	4	2	0.22222	0.88889	0.00649	0.00662
	Total	7	4	0.34762	1.29524	0.00945	------

The total no of segregating sites (S) for Population wise Measures of DNA sequence polymorphisms and tests of neutrality was varied from 16 (RNAR 8) to 5 (UDPGDB3). Number of haplotypes also high 5 in RNAR 8 where for the rest two locus were 3 (UDPGDB3) and 4 (SUSY8) respectively. Haplotypes diversity and nucleotide diversity value were 0.53216 and 0.01299 which is very high value than other two loci. Individually *Vigna hainiana* showed maximum value in all respect for all the three loci (Table 15a, 15b and 15c).

4.10 Gene Tree

The Minimum Evolution tree (Fig. 10a) for the locos BV165289 had *silvestris* BB2638 a separate branch and *silvestris* BBL 63 2K and *silvestris* BBL 63 2K were much closed to each other. Neighbor Joining tree (Fig.10b also gave the same pattern as ME tree only species were different.

According to CLUSTERX (1.83) MULTIPLE SQQUENCE ALIGNMENT in the locus BV165289 there was a deletion of a single nucleotide in *silvestris* BBL63-2K, BBL55-2K, BBL76-2K and mungo BBD 2649. There was an insertion of a single nucleotide for *hainiana* BB 2649.

The minimum evolution tree (Fig.11a) for the locus OLM1 there was two main branches. In cluster I silvestris BBL76 2K was connected with *mungo* Vanban 2 with a bootstrap value of 93%. On another cluster, mungo BBD-08-01B, mungo BBD-14-01B and mungo BB-03-09 were lying in a same group with bootstrap values of 100 %. In

cluster II mungo IPU 99 was separate from all other with a bootstrap value of 30%. Neighbor Joining tree (Fig.11 f) also gave the same pattern as ME tree. In Maximum parsimony (MP1) number of nucleotide insertion was three and the nucleotide was 'A' where as MP 2 number of insertion two ('T') in MP 3 and in MP 4 four ('A'). For the locus SHMT1 the cluster was divide into two main clusters one was having only one species (silvestris BBL 40 2K) and another cluster containing rest all the accessions except silvestris

Legend (Figure 11)

Figure 11: Gene trees showing minimum evolution and neighbour joining tree for locus BV16 (a) ME, (b)NJ

BV165289 Locus.

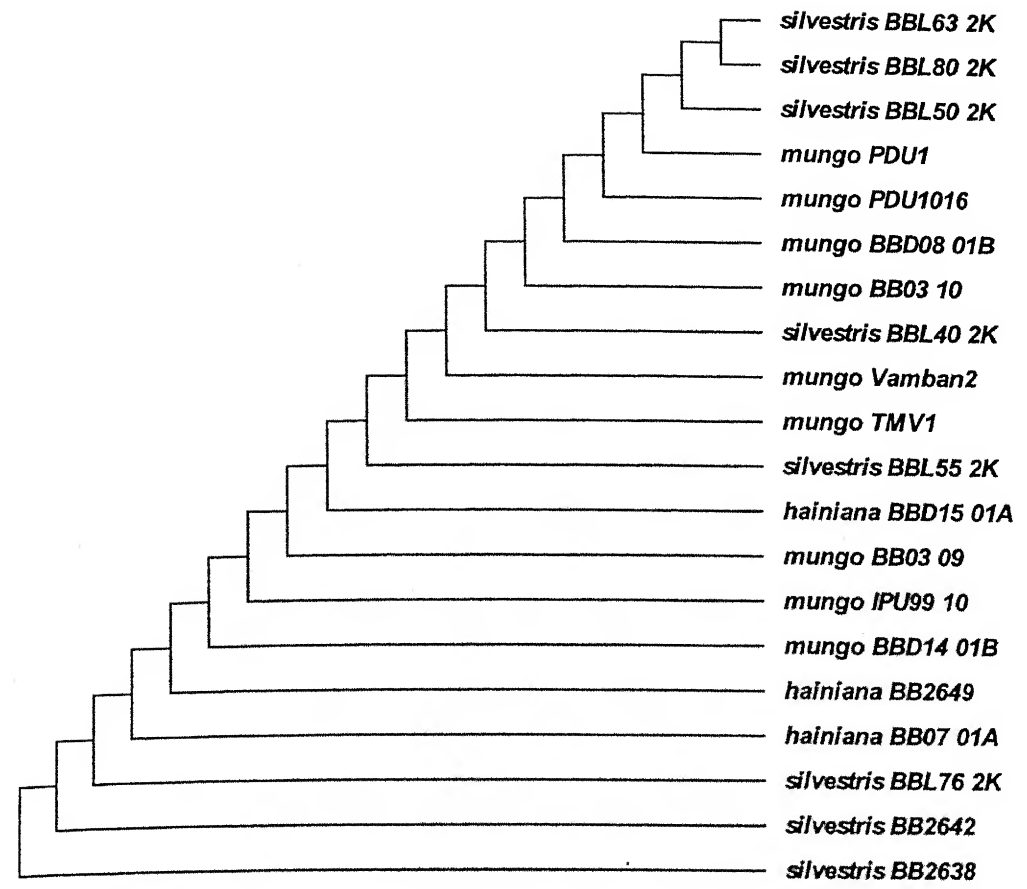


Figure 10(a) ME

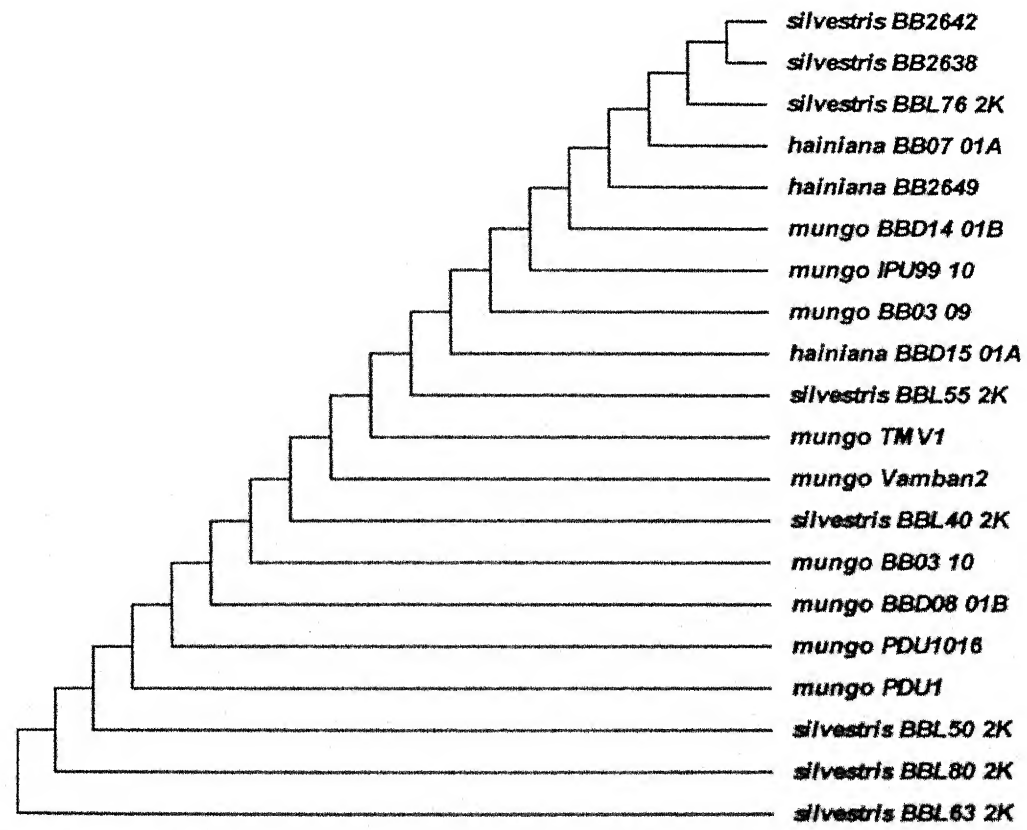


Figure 10(b) NJ

Legend (Figure 12)

Figure 12: Gene trees showing neighbour joining minimum evolution and maximum parsimony tree for locus OLM1 (a-f)

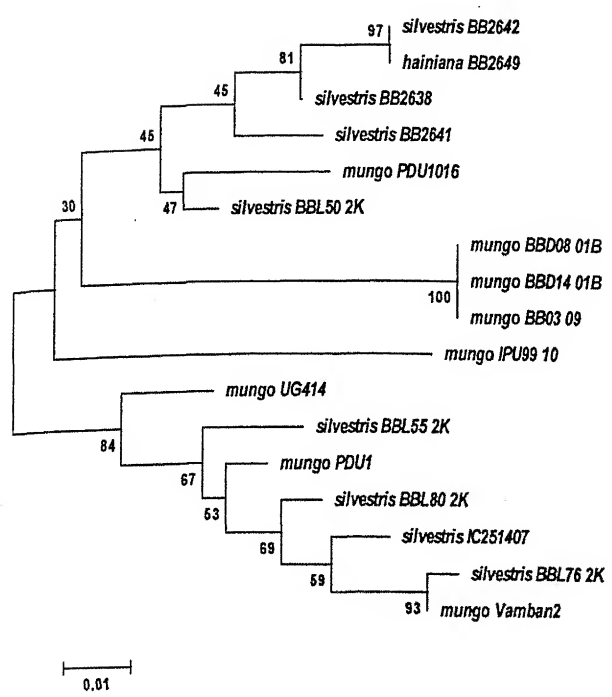
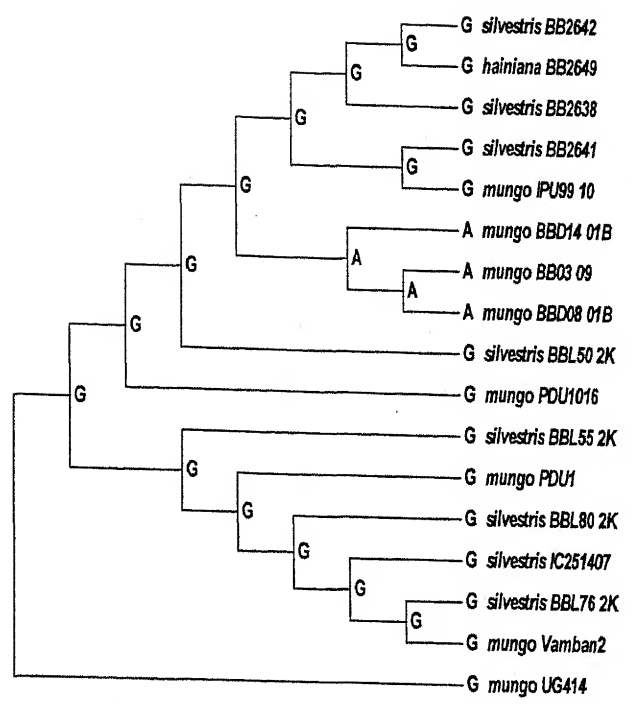
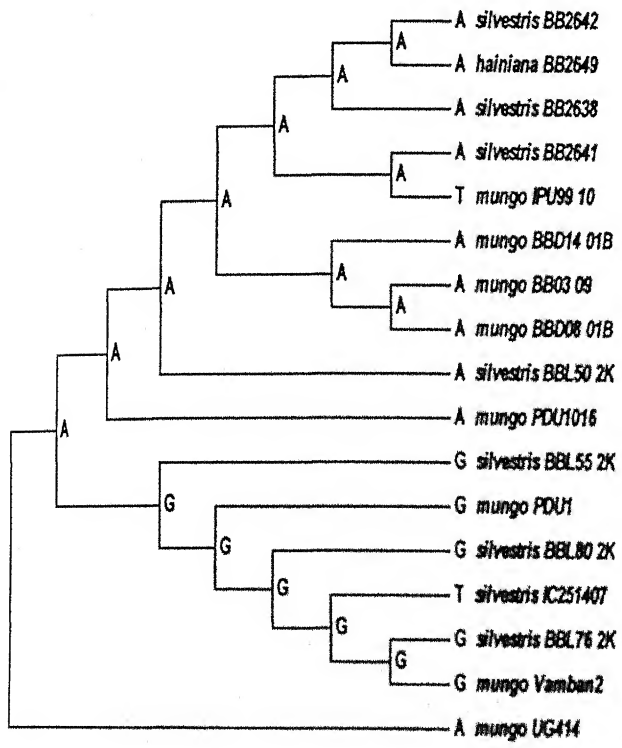


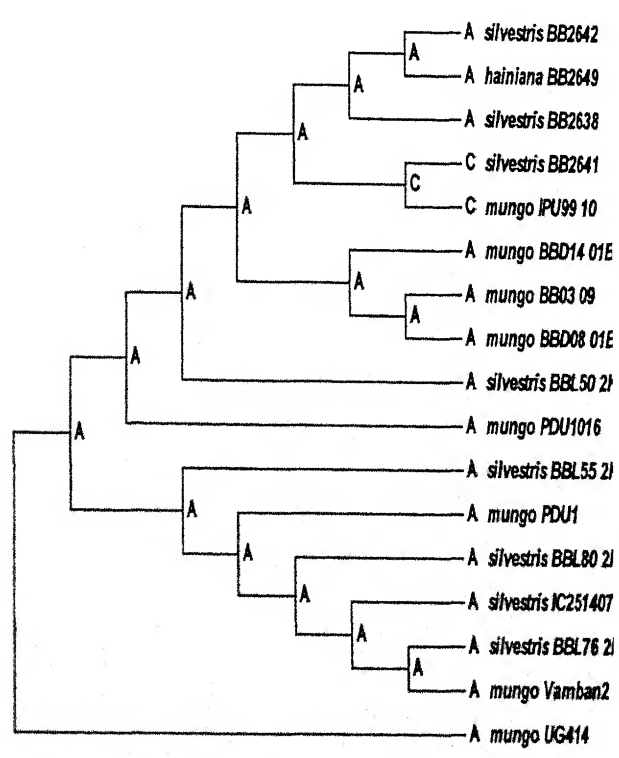
Figure 11(a) ME



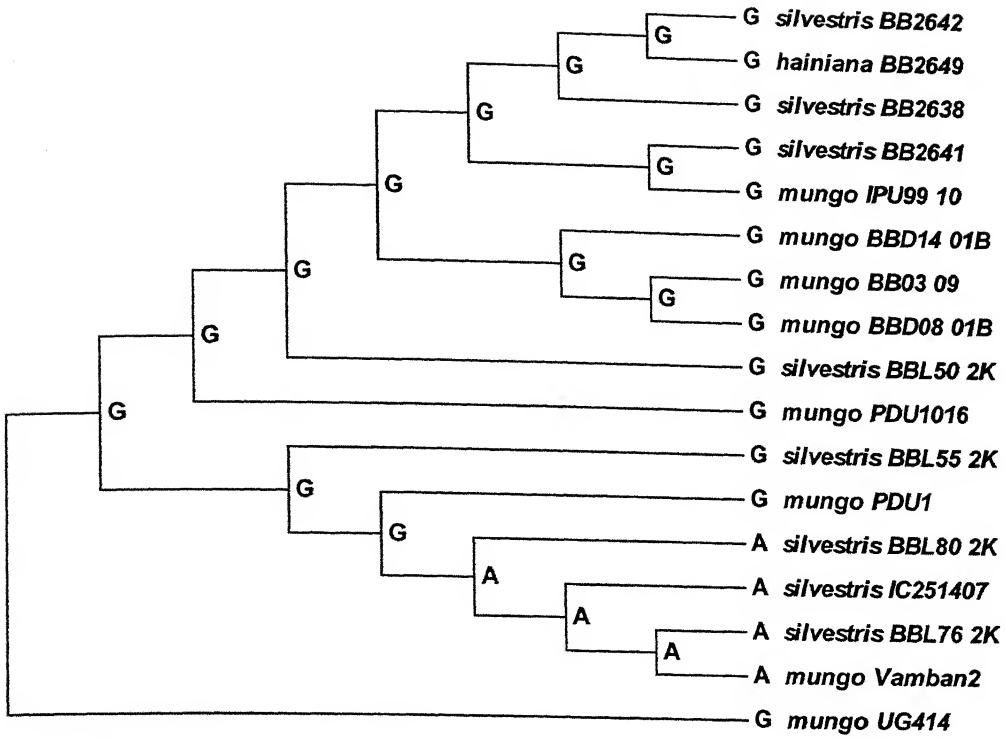
(b) MP1



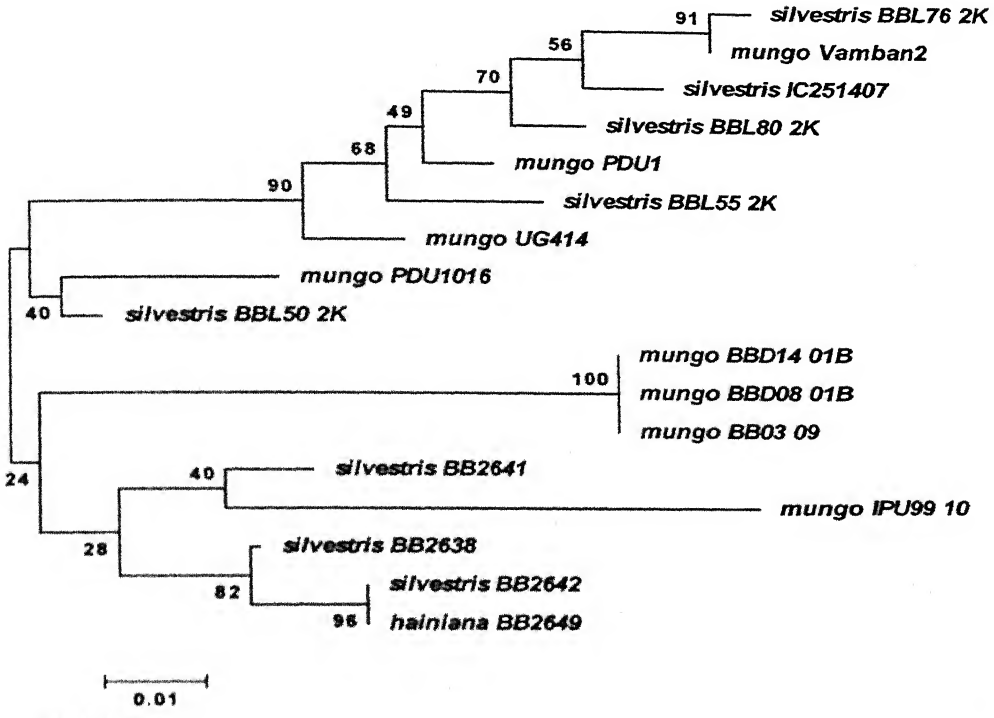
(c) MP 2



(d) MP 3


G silvestris BB2642
G hainiana BB2649
G silvestris BB2638
G silvestris BB2641
G mungo IPU99 10
G mungo BBD14 01B
G mungo BB03 09
G mungo BBD08 01B
G silvestris BBL50 2K
G mungo PDU1016
G silvestris BBL55 2K
G mungo PDU1
A silvestris BBL80 2K
A silvestris IC251407
A silvestris BBL76 2K
A mungo Vamban2
G mungo UG414

(e) MP 4


91
56
70
49
68
90
40
100
24
40
28
82
96
silvestris BBL76 2K
mungo Vamban2
silvestris IC251407
silvestris BBL80 2K
mungo PDU1
silvestris BBL55 2K
mungo UG414
mungo PDU1016
silvestris BBL50 2K
mungo BBD14 01B
mungo BBD08 01B
mungo BB03 09
silvestris BB2641
mungo IPU99 10
silvestris BB2638
silvestris BB2642
hainiana BB2649
0.01

(f) NJ

IC251407. Neighbor Joining tree (Fig. 12b) also gave the same pattern as ME tree only species were different.

Analysis of CLUSTERX in three species (*silvestris* BB 264, BBL80-2K and *mungo* PDU 1) there is single nucleotide difference and SNP found in *silvestris* BB2641 and *mungo* IPU 99/10. There was several nucleotide differences between accessions.

Neighbor Joining tree and minimum evolution (ME) tree for the locus SHMT1 was mirror image of one another. There were two main cluster, one cluster consist only one accession *silvestris* BBL40-2K and another cluster consist rest of the samples. In CLUTERX deletion of single nucleotide found in *silvestris* BBL40-2K, BBL50-2K and *mungo* BB03-09 and insertion of single nucleotide in *silvestris* IC251407, BBL40-2K, BBL80-2K and *mungo* IPU 99/10. Single nucleotide polymorphism (SNP) found in *silvestris* IC 251407 and BBL40-2K.

Maximum evolution (ME) tree for the locus SUSY 8 had main two clusters. One consist only one accession *silvestris* IC251407 which is diverse from all others accessions. Cluster II divided into three sub cluster. Sub cluster one no groping found but in subcluster II *hainiana* BB0701A and *hainiana* BBD15-01A grouped together. Neighbor Joining tree (Fig. --) also gave the same pattern as ME tree only species were different. Analysis of CLUSTERX there was a single nucleotide deletion and insertion found in *hainiana* BB07-01A and *hainiana* BB2649 respectively. Single nucleotide polymorphism (SNP) was detected in *silvestris* BB76-2000 and IC 251407.

Neighbor Joining tree and minimum evolution (ME) tree for the locus UDPGDB3 found only two cluster. *Hainiana* KP3 Raj, IC251377 and BB07-01A were grouped together and rest all the accessions in another group. For the Maximum parsimony no such insertion was found. In CLUSTERX no deletion was found only insertion was found in *silvestris* BB2642. Single nucleotide polymorphism (SNP) was found only in *hainiana* BB2649.

Legend (Figure 13)

Figure 13: Gene trees showing neighbour joining and minimum evolution tree for locus SHMT (a and b)

Legend (Figure 14)

Figure 14: Gene trees showing neighbour joining, minimum evolution and maximum parsimony tree for locus SUSY (a -c)

SHMT Locus

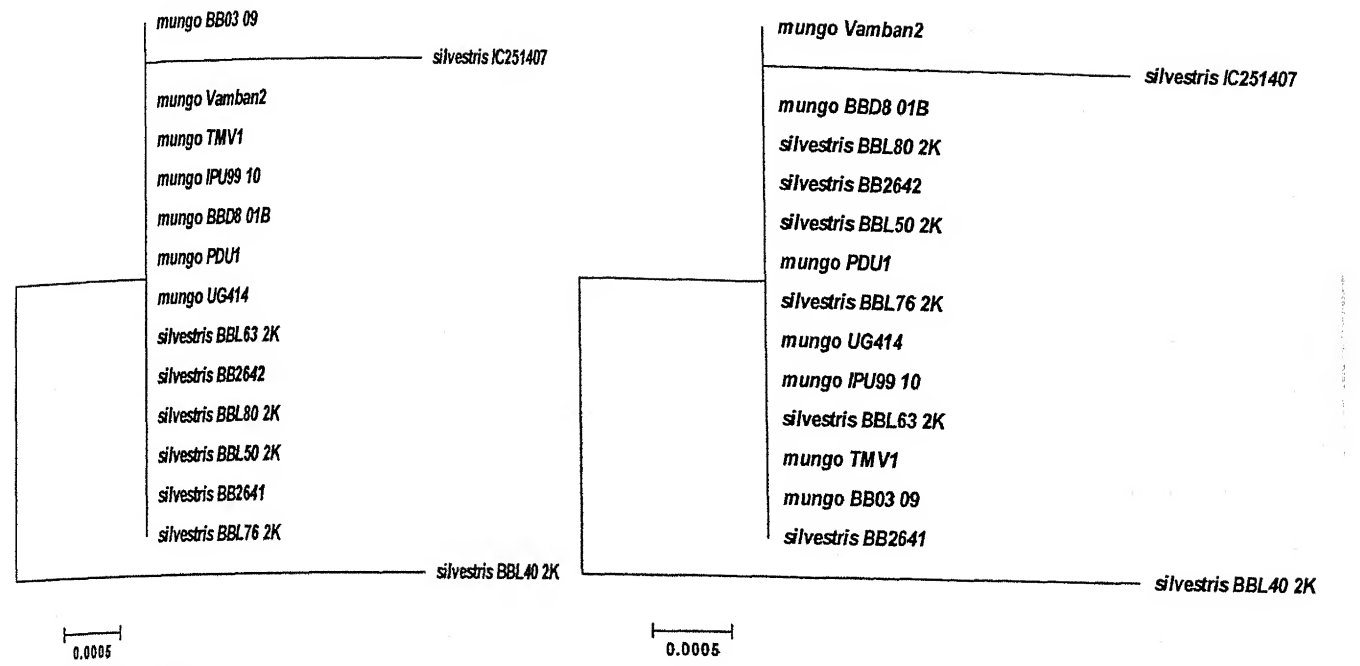


Figure 12(a) ME (b) NJ

SUSY Locus

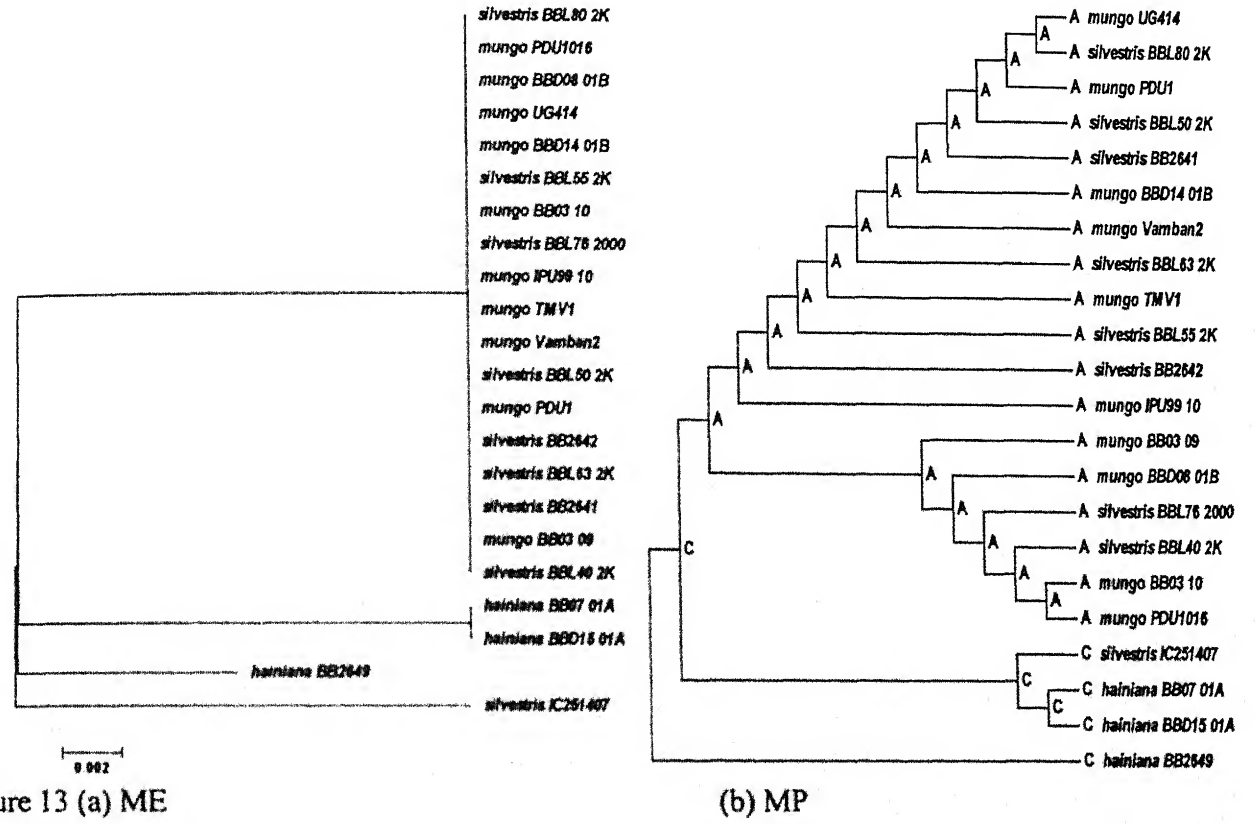
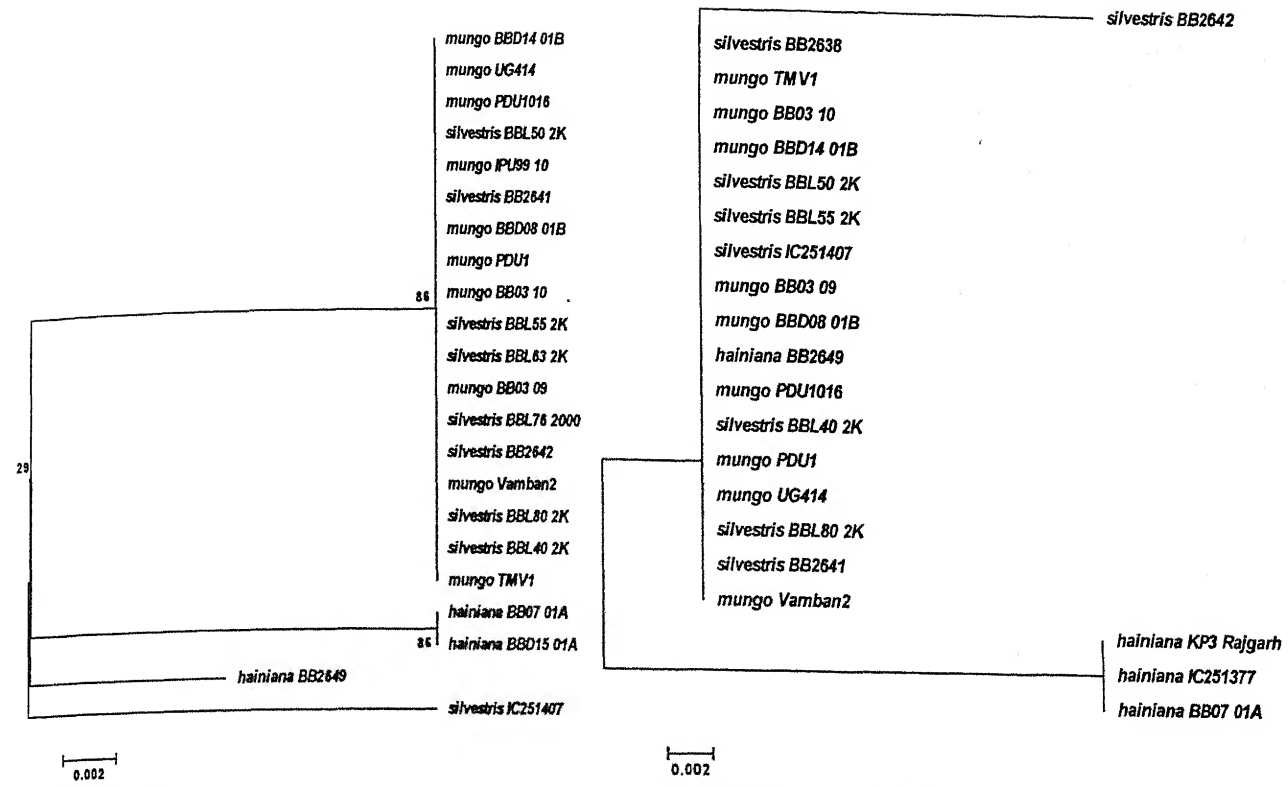


Figure 13 (a) ME (b) MP

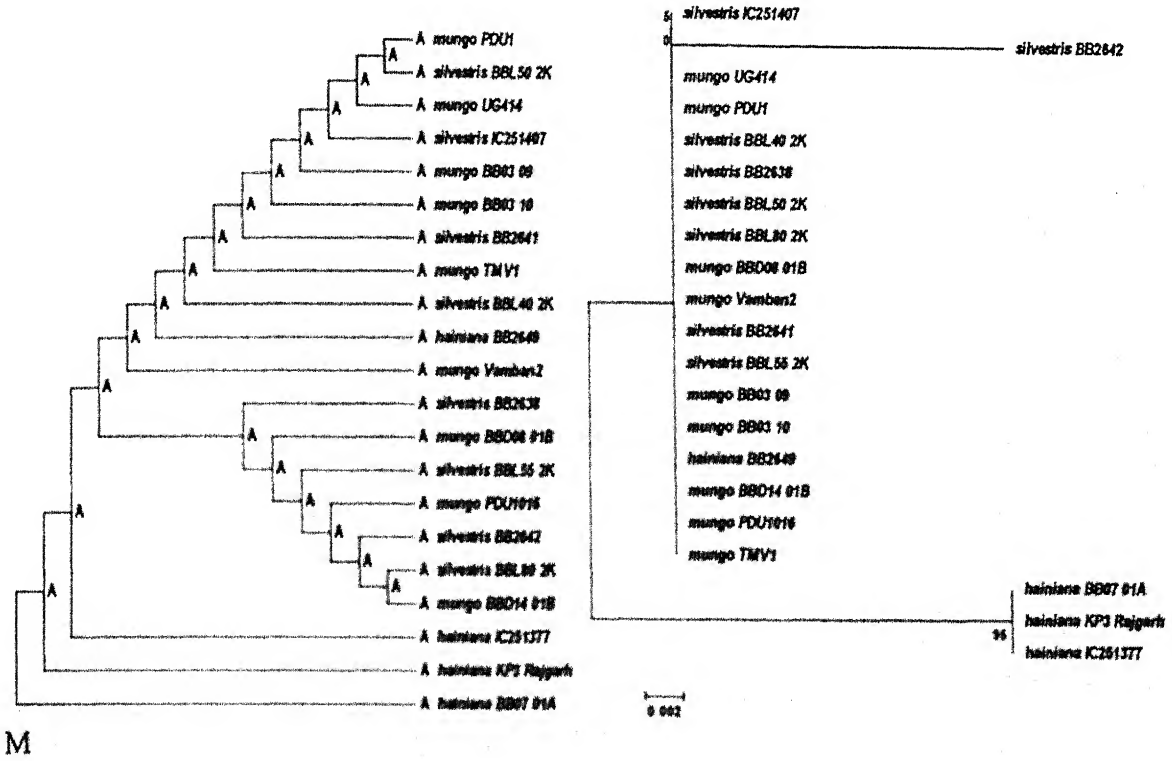
Legend (Figure 15)

Figure 15: Gene trees showing neighbour joining minimum evolution and maximum parsimony tree for locus UDPGDB3 (a-c)



(c) NJ (SUSY)

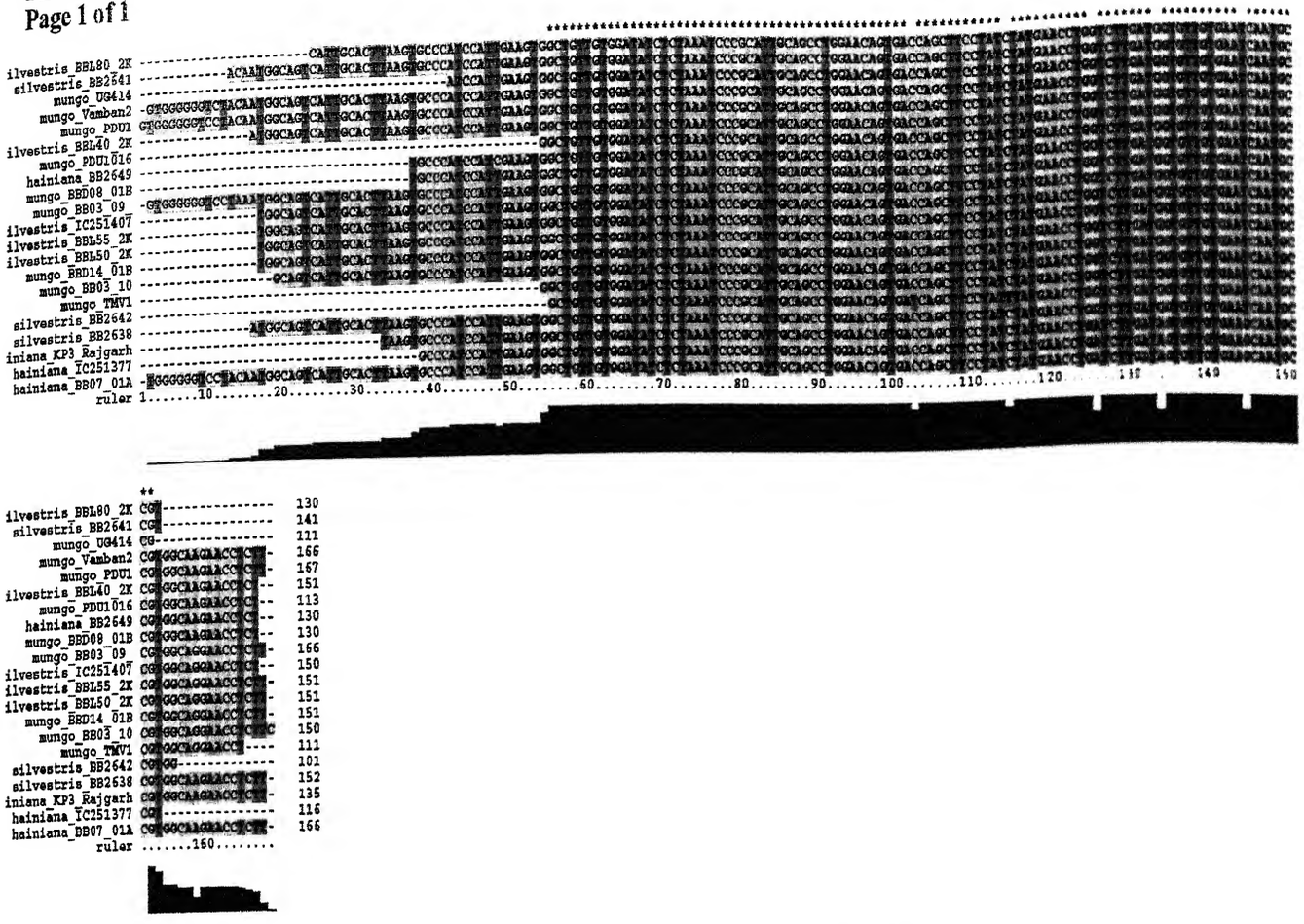
Figure 14 (a) ME (UDPGDB3)



(b) M

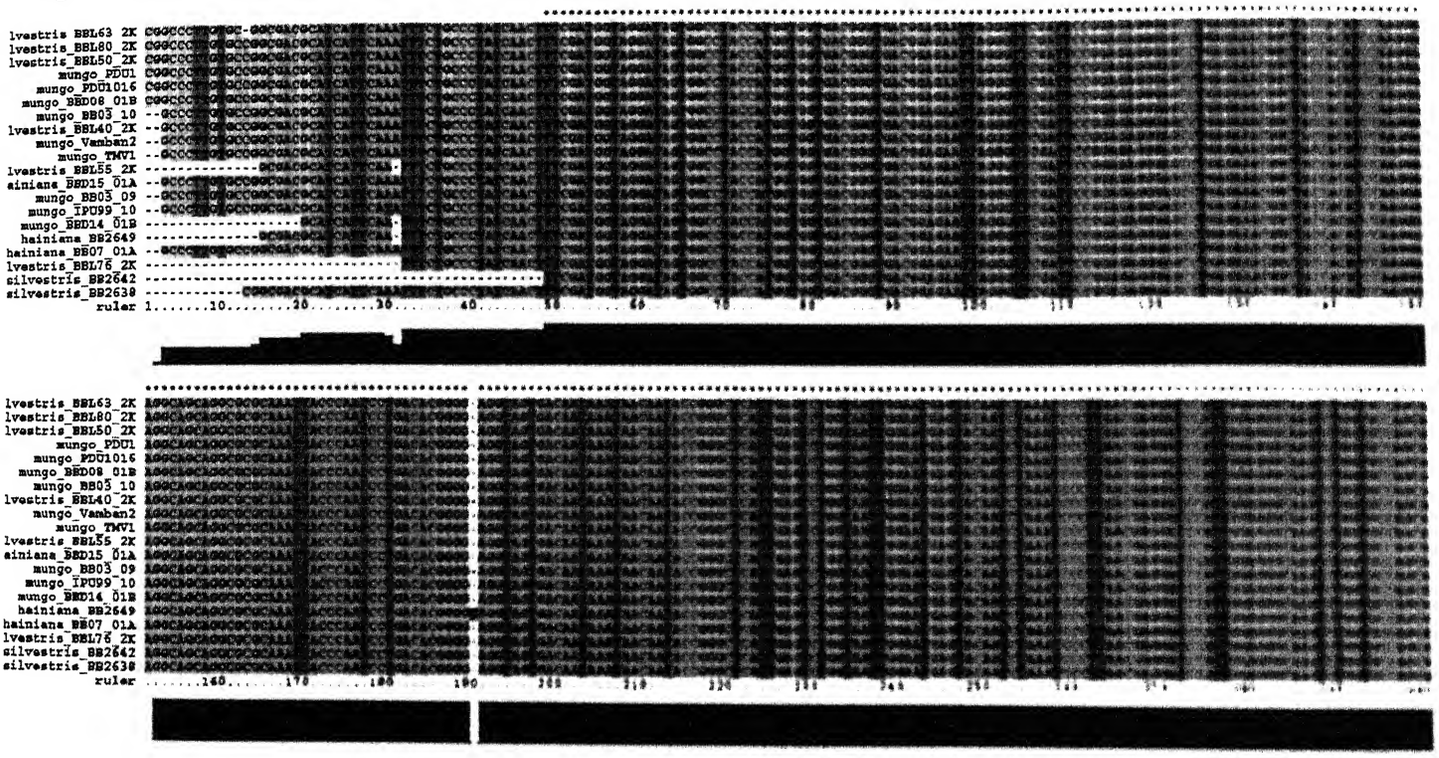
CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

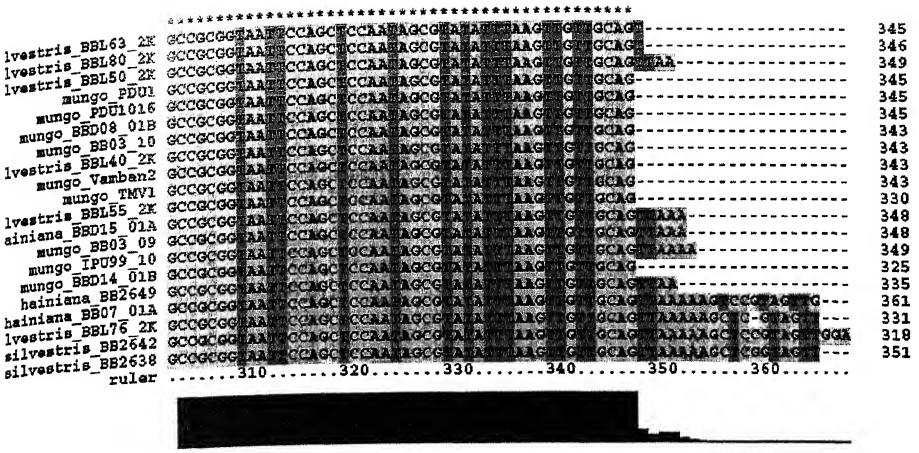
File: C:Documents and SettingsK.V.BhatMy DocumentsKoleKOLE SEQKUDPGDBD.ps Date: Thu Jun 04 10:21:16 2009
Page 1 of 1



CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

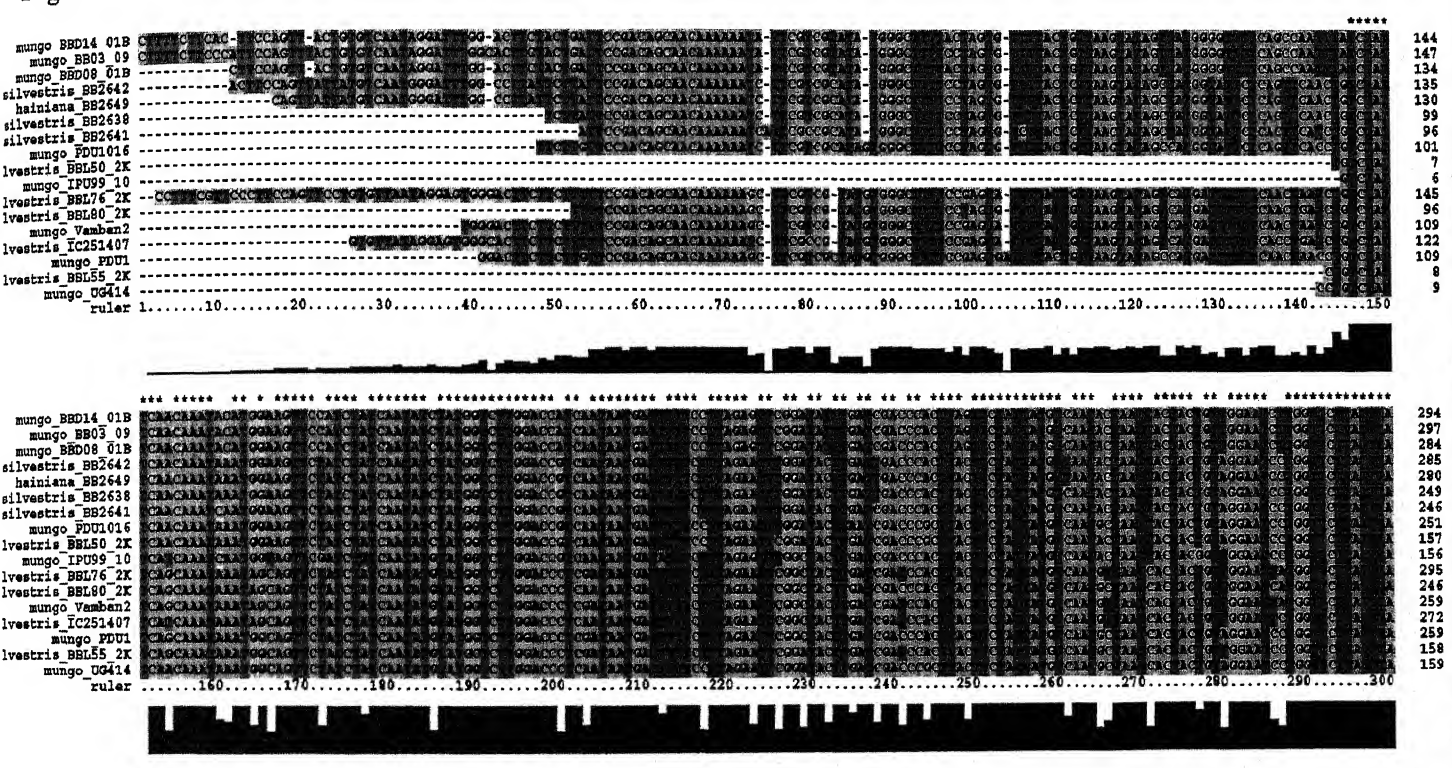
File: C:Documents and SettingsK.V.BhatMy DocumentsKoleKOLE SEQKBV16D.ps Date: Thu Jun 04 09:48:41 2009
Page 1 of 2

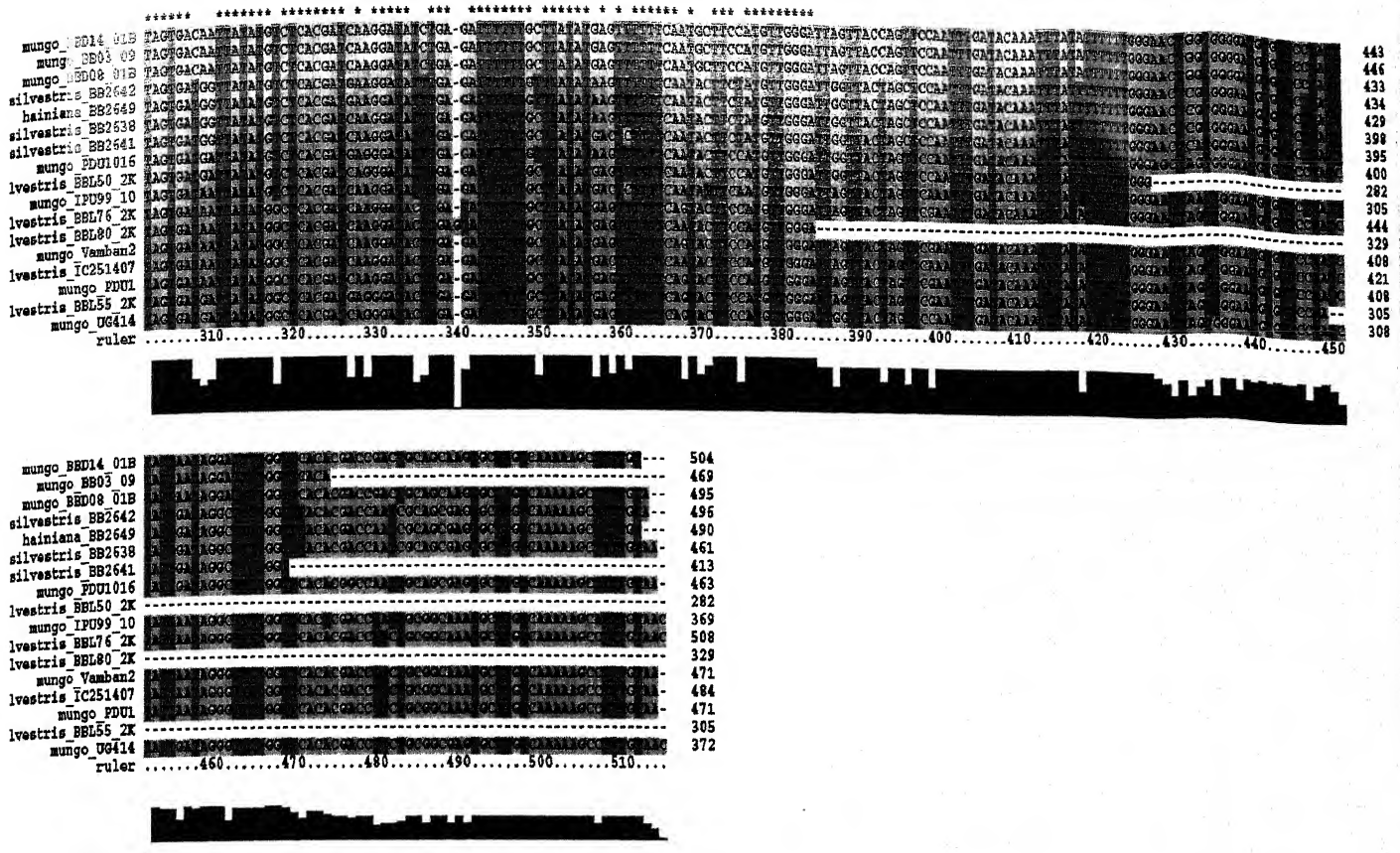




CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

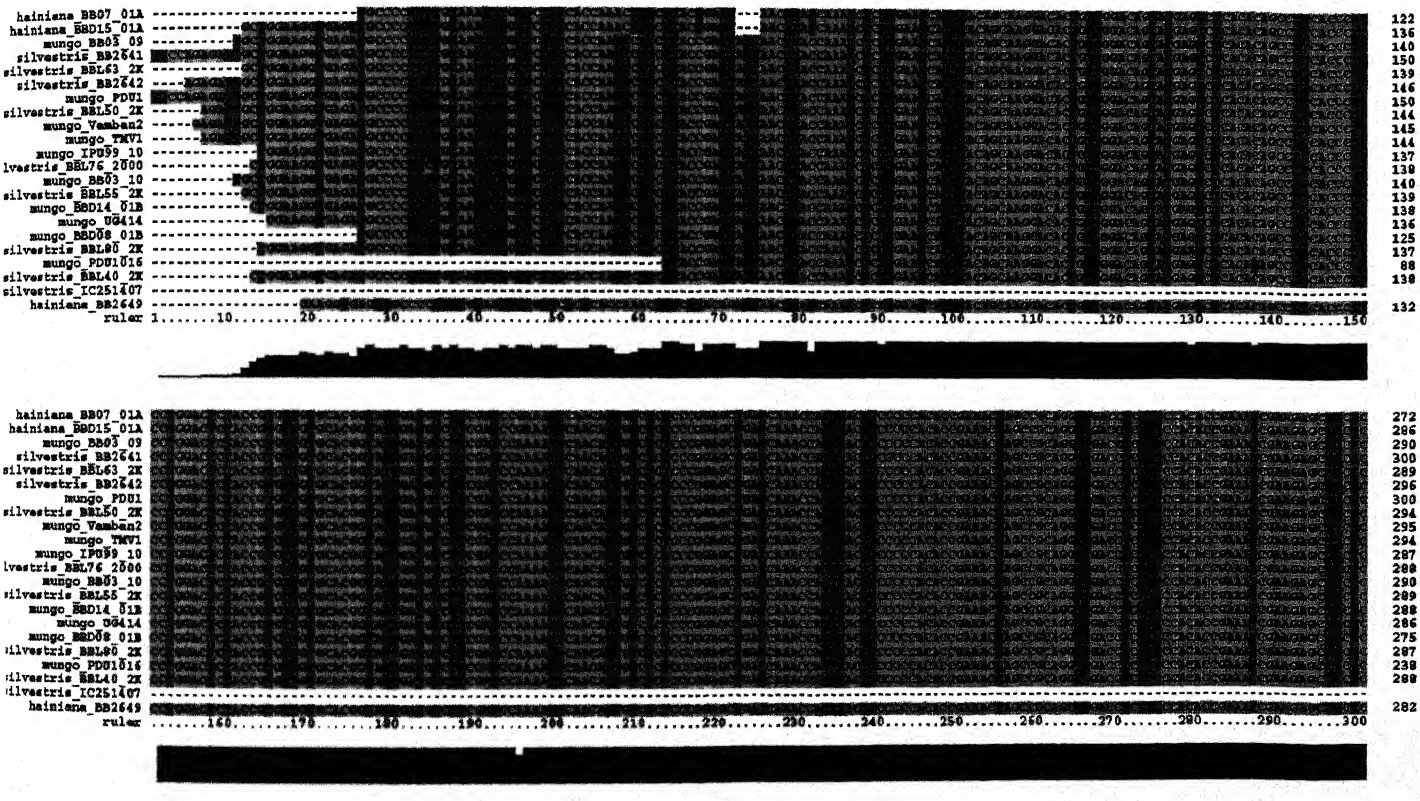
File: C:Documents and SettingsK.V.BhatMy DocumentsKoleKOLE SEQKOLMD.ps Date: Thu Jun 04 10:17:40 2009
Page 1 of 2

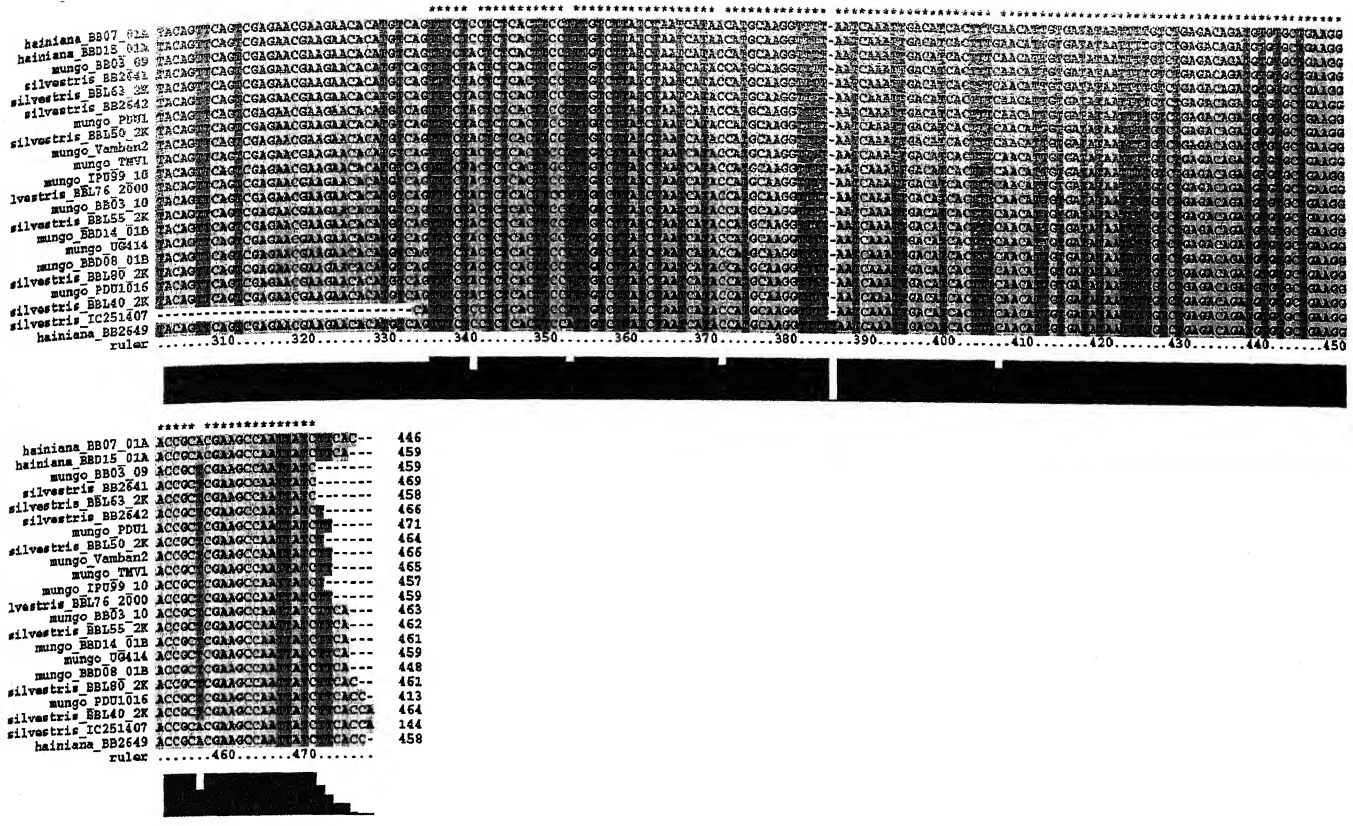




CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

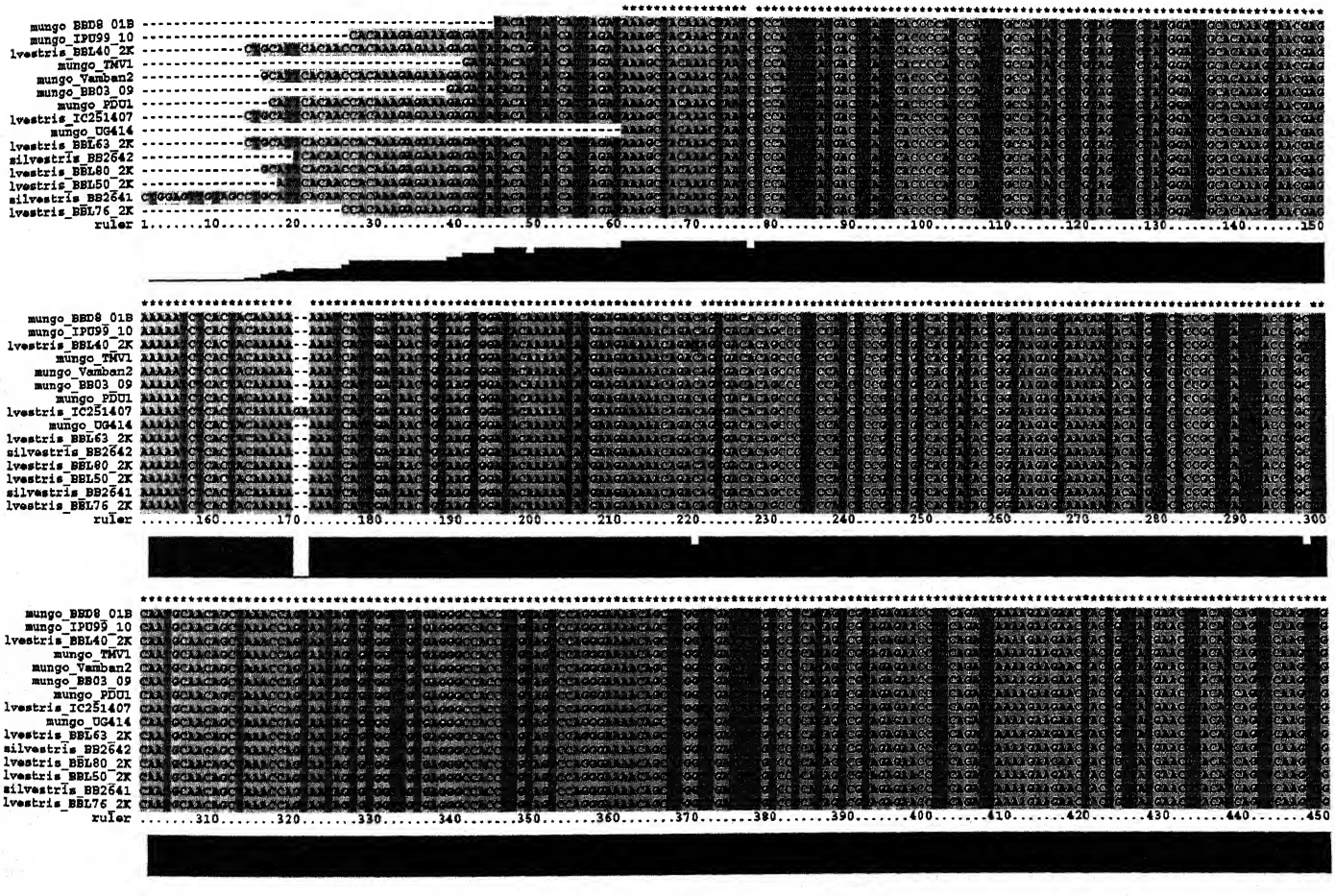
File: C:Documents and SettingsK.V.BhatMy DocumentsKoleKOLE SEQKSUSYD.ps Date: Thu Jun 04 10:20:20 2009
Page 1 of 2

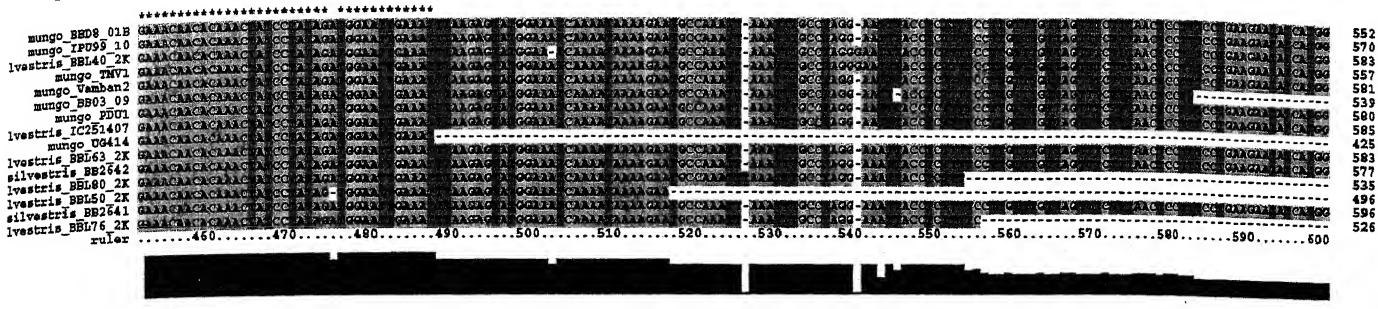




CLUSTAL X (1.83) MULTIPLE SEQUENCE ALIGNMENT

File: C:Documents and SettingsK.V.BhatMy DocumentsKoleKOLE SEQKSHMTD.psDate: Thu Jun 04 10:18:57 2009
Page 1 of 2




mungo_BBD8_01B
mungo_IPU95_10
lvestris_BBL40_2K
mungo_TMV1
mungo_Vamban2
mungo_BB03_09
mungo_PDU1
lvestris_IC251407
mungo_UG414
lvestris_BBL63_2K
silvestris_BB2642
lvestris_BBL80_2K
lvestris_BBL50_2K
silvestris_BB2641
lvestris_BBL76_2K
ruler
.....460.....470.....480.....490.....500.....510.....520.....530.....540.....550.....560.....570.....580.....590.....600
552
570
583
557
581
539
580
585
425
583
577
535
496
596
526


mungo_BBD8_01B
mungo_IPU95_10
lvestris_BBL40_2K
mungo_TMV1
mungo_Vamban2
mungo_BB03_09
mungo_PDU1
lvestris_IC251407
mungo_UG414
lvestris_BBL63_2K
silvestris_BB2642
lvestris_BBL80_2K
lvestris_BBL50_2K
silvestris_BB2641
lvestris_BBL76_2K
ruler
.....610.....620...
573
593
605
572
602
539
599
606
425
604
596
535
496
613
526

DISCUSSION

Advances in cellular and molecular biology have provided new tools like marker aided selection, which increases the efficiency of breeding programmes especially disease resistance breeding. In conventional methods, selection of genotype relied on field or glass house screening. It may also require the use of controlled envioronment, when the disease development is temperature sensitive as that of yellow mosic virus. Hence, the screening process is complex and time consuming. But in molecular marker assisted selection, once a gene of interest is tagged with molecular marker, selection for that gene can be made based on the marker. In present study, an attempt was made to identify the RAPD marker associated with MYMV resistance, using bulk segregant analysis. One of the most time consume requirement in marker development is the need to screen entire mapping population, suggest that testing the entire population is required only when polymorphism between the bulks are detected. This results in a considerable saving of time particularly when used PCR based technique such as RAPD (Williams et al. 1990).

A total of sixty six random decamers were surveyed for identification of polymorphic markers between the parents and twenty four produced specific band for resistance parent which were absent in susceptible parent. However the parents were chosen at Allahabad according to there specific characters such as resistance for MYMV and it were clearly distinguished there. But at Delhi all the F_2 individuals were found to be susceptible along with the parents except some individuals (Plant no 47, 65, 80 and 90) according to interaction phenotypic scale. Due to some unavoidable conditions and the total number of F_2 individuals was found to be very less. The climatic conditions in which the F_2 individuals were grown was not appropriate. Either due to pathogens found in Delhi or the environmental conditions the number of F2 was reduced to 46. These small number of F2 population were not sufficient to generate a molecular map. Hence our study was further extended to find the closest wild species related to our crop which are resistance to some disease so that diversity analysis and gene flow can be studied. Further we tried to search for the single nucleotides which made differences.

Wild relatives of crops species are source of important genes for agriculture and genetic diversity measured at the biochemical and DNA level is generally greater in wild species than there related cultigen (Hallan, 1984; Xu et al., 2000). Intensive modern breeding has contributed to a narrowing of crop genepools as a few improved cultivers dominate large areas (Ladizinsky, 1985). Due to recent development of gene transfer technology, genes from cross incompatible wild species can be use for breeding (von Bothmer & Seberg, 1995). Therefore, evaluation of a broad array of wild species is an important approach to seeking genes which rare or absent in cultigens.

Wild Relatives as Genetic Resources

Fuzi and Miyazaki (1987) reported an accession (TC1966) of *V. radiata* var. *sublobata* that showed perfect resistance against azukibean weevil (*Callasabruchus chinensis*). The resistance was found to control by a single dominant (Kitamura et al, 1988). Fuzi et al (1989) further found that TC1966 is completely resistant against *C. maculates, C. phaseoli* and *Zabrotes subfasciatus*. Tomooka et al (1992) developed a bruchid resistant mungbean line in Thailand by using TC1966 as a gene source. In addition to bruchid resistance, high resistance to yellow mosaic virus (Singh and Ahuja, 1977), high methionine content in seeds (Babu et al, 1988), higher photosynthetic activity and tolerance to drought ((Ignacimuthu and Babu, 1987), higher tolerance to saline and alkaline soils (Lawn et al, 1988) have been reported for *V. radiata* var. *sublobata*. In comparison to *V. radiata* var. *sublobata* there are very few studies of evaluation of *V. mungo* var. *silvestris* as genetic resource. However, this variety is cross compatible with *V. mungo* (Miyazaki et al, 1984). Therefore, further collection and evaluation *V. mungo* var. silvestris is necessary for genetic upgradation of *V. mungo*.

Information on the levels and distribution of genetic diversity of any plant species may contribute to the knowledge of their evolutionary history and potential, and is critical to their conservation and management (Hamrick and Godt, 1996). Several markers may be used to identify and assess the genetic diversity and phylogenetic relationship in plant genetic resources. The traditional method based on morphological traits requires

extensive observation of mature plants and can not serve as unambiguous markers because of environmental influences (Wrigley *et al.*, 1987).

With the advent of molecular markers, a new generation of markers has been introduced over the last two decades, which has revolutionized the entire scenario of biological sciences. DNA-based molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, physiology, embryology, genetic engineering, etc. They are no longer looked upon as simple DNA fingerprinting markers in variability studies or as mere forensic tools. Ever since their development, they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis. The discovery of PCR (polymerase chain reaction) was a landmark in this effort and proved to be a unique process that brought about a new class of DNA profiling markers. This facilitated the development of marker-based gene tags, map-based cloning of agronomically important genes, variability studies, phylogenetic analysis, synteny mapping, marker-assisted selection of desirable genotypes, etc. Thus giving new dimensions to concerted efforts of breeding and marker-aided selection that can reduce the time span of developing new and better varieties and will make the dream of super varieties come true. These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analysed objectively. In this article, DNA markers developed during the last two decades of molecular biology research and utilized for various applications in the area of plant genome analysis are reviewed.

A total of 375 individuals (15 seeds of 26 accessions of 3 species) of vigna showed the polymorphism within accessions and within groups using STMS analysis. The ten polymorphic primer pair used to study in which two loci were found to be highly polymorphic with 5-4 alleles. V. hainiana and V. silvestris both showed maximum number of polymorphism. The product size ranged from 150bp to 320 bp. This showed the efficiency of the marker used and the fingerprinting methods. However, compared with the use of morphological markers, the results obtained using microsatellite markers are more stable because the repeat sequences are hardly affected by factors such as the environment, sampling time, which part is sampled and especially the choice of external factors during evolution.

Microsatellite analysis needs little DNA but, detects much polymorphism, gives reliable results and is simple to operate. Therefore, STMS is a convenient and reliable tool to quickly identify the genetic diversity of large numbers of germplasm (Hong- Liang et al., 2004). Specific markers like STMS (sequence-tagged microsatellite markers) or STS markers have proved to be extremely valuable in the analysis of gene pool variation of crops during the process of cultivar development, and classification of germplasm. These markers are extremely sensitive and can detect allelic variability during cultivar development. STS markers specific to chloroplast or mitochondrial DNA have been useful in providing seed and pollen specific markers which can be utilized for the detection of length variation at multiple physically linked sites and may be used to provide haplotype data and thus genotypically unique individual plants.

Population structure is the consequence of multiple events, starting from the modalities of domestication, the structure being further shaped by modern breeding. The lack of clear population differentiation, high heterozygosity and gene flow, low fixation indices all point towards a high rate of germplasm migration across geographic regions in the country. There is a considerable exchange via buying, sharing of cultivars across mungo growing belts as well as introduction and spread throughout the country. Analysis of molecular variance, low FST value, cluster analysis and high values for estimates of Nm indicated presence of substantial gene flow between the cultivars from different regions, possibly due to cultivar exchange followed by gene introgression by crossing and selection.

The POPGENE analysis was done for the populations as well as for the groups. Groups were classified into three. Group I was formed of V. mungo. Group II was of V. silvestris and group III was of V. hainiana. Total number of alleles, effective number of alleles and Shannon's Index were calculated for 25 accessions as well as for three groups. The effective number of alleles varied from 3.0 to 1.7. Shannon's index was maximum in silvestris BB2641. In three groups Shannon's index was highest in V. m. silvestris which is a wild relative of Vigna mungo.

The observed heterozygosity and expected heterozygosity were also calculated for population and groups. The mean values of observed and expected heterozygosity for 25 populations were 0.084 and 0.335 respectively. Since observed was less than expected the

values suggested that there was no cross pollination also with in populations. For groups the observed was less than expected suggesting self pollination nature of urdbean.

Nei's original measure of genetic distance was calculated for population as well as for groups. In group I the closest relation was found in mungo BBD08-1B, IPU99/10, TMV1 and PDU1. For group II the closest relationship was found in between V. silvestris BBL63-2Kand BBL80-2K. IN group III the accession BB07-2k and BBD15-2K was the closest. Population dendrogram was made by Nei's genetic distance. All 25 accessions were clustered. Dendrogram were clearly clustered no inter groping was found in this dendrogram. Dendrogram were clearly clustered no inter groping was found in this dendrogram. Two main clusters were formed with three species. Cluster I consist of V. mungo and V. silvestris accessions and cluster II was V. hainiana accessions. Cluster I again divide into two, on which one contain V. mungo with some accessions of V. silvestris. Another one consists of four V. r. silvestris accessions which were closely relative.

Fixation statistics were produced for individual SSRs and groups of germplasm. By using 1000 permutations significance of the estimates were obtained. The percentage of variation within populations (16.03%) followed by 39.13% among populations within group and 44.84% among groups. Average fixation indices were FSC: 0.710, FST: 0.840 and FCT: -0.84 indicative of high level of outcrossing and gene flow and no geographic isolation at any hierarchical level. High levels of gene flow via germplasm exchange may have caused orchard stands in the northern plains and related regions to be genetically connected with little genetic differentiation.

The population differentiation value (Fst) was used to determine the amount of geneflow (Nm). The values were calculated separately for populations and for groups. Fst value is inversely proportional to Nm. The Nm value for the populations was found to be negligible, suggesting no gene flow within populations. For the groups, Nm value for V. mungo was 0.4647 which was more than both V. r. silvestris and V. hainiana. Since V. mungo is a cultivated it showed the highest gene flow within. The Ewen-Watterson test

for neutrality was done using 1000 simulated samples for the 10 loci all were neutral to selection pressure.

The numbers of segregating sites (S), observed nucleotide diversity per site between any two sequences assuming that the sample is random (p), number of haplotypes (H), haplotype diversity (Hd), and average number of pairwise nucleotide differences within population (K) were calculated using DnaSP. Tajima's D correspond to positive natural selection whereas negative values correspond to negative or purifying selection. The genetic differentiation among the parasite populations was calculated in terms of fixation index (Fst) that estimates diversity within a subpopulation with respect to total genetic

diversity. In addition, average number of pairwise nucleotide differences (Kxy), nucleotide substitution per site (Dxy), and net nucleotide substitution per site (Da)

between populations were also calculated. The above parameters were also estimated on DnaSP. Phylogenetic analysis was performed by neighbour-joining (NJ) method with Kimura 2-parameter distance matrix inMEGA version 3.0

For the sequencing of the samples sequence tag microsatellites (STS) marker was used. Mostly haplotype diversity, Tajima'sD observed pairwise nucleotide diversity was calculated. Positive value for locus OLM1 of tajima's D indicates the positive neutral selection. Measure of DNA sequence polymorphism and neutrality test table(table 16) indicates that OLM1 marker was more informative than other markers. Fst values for Loci SUSY was highest 0.628 and Nm was calculated to be maximum 7.11 in the locus OLM1 indicated that the geneflow was very high mainly suggesting some cross pollination in the crop.

Inter population nucleotide difference (Kxy) varied from 0.0235 (between silvestris and mungo) in locus UDPGDB3 to 26.639 (between mungo and hainiana) in OLM1. Similarly, the average number of nucleotide (Dxy) and net nucleotide (Da) substitutions per site between populations ranged from 0.00088 (SHMT1) to 0.09146 (OLM1) respectively. Inter-population comparison of the total (TM) versus shared (SM) number of mutation showed only on locus OLM1 (29 of 53 mutations were shared) (Table 17). Gene flow was varied from 0.31 (SUSY8) to 8.83 (OLM1) on basis of

haplotype data information (Table 18). Based on sequence data information it was found 0.15 (SUSY8) to 7.11 (OLM1). Total number of segregating sites (S) for Population wise Measures of DNA sequence polymorphisms and tests of neutrality was varied from 16 (RNAR 8) to 5 (UDPGDB3). Number of haplotypes also high 5 in RNAR 8 where for the rest two locus were 3 (UDPGDB3) and 4 (SUSY8) respectively. Haplotypes diversity and nucleotide diversity value were 0.53216 and 0.01299 which is very high value than other two loci. Individually vigna hainiana showed maximum value in all respect for all the three loci. Above information showed the relation between cultivate and wild species. In the all aspect mungo and hainiana were differ from each other. So for the future studies wild species can be used for breeding and crop improve programme.

The gene trees were prepared for the locus SHMT, SUSY8, BV16 UDPGDB3 and OLM1. Three types of trees were discussed, Neighbour Joining, Minimum Evolution and Maximum Parsimony. For the locus SHMT, SUSY8 BV16 and UDPGDB3 neighbour joining tree and minimum evolution tree were almost similar in the pattern of clustering. Except for the arrangement of some species both the trees showed no variation in the cluster formation. Maximum parsimony tree showed the variation in the single nucleotide. Instead of 'T' either 'A' or 'G' was inserted. Similarly for the locus SUSY and SBV16, the cluster formation for neighbour joining and minimum evolution was similar. The maximum informative locus was OLM1.

The alignment of the species for the three loci showed the single nucleotide insertions and deletions. Cluster formation was done on the basis of this single nucleotide polymorphism. Very less number of mutations was observed hence variation was very less. Comparing between five loci OLM1 showed more information than SHMT, UDPGDB3, SUSY and SBV16 hence these locus gave more information.

SUMMARY

SUMMARY

Pulses are unique crops that are a rich source of vegetable protein and have in-built mechanism to fix atmospheric nitrogen. India is a major pulse growing country in the world, with about 37% of area and 28% of the production. Besides the immense importance with regards to nutritional and food security, pulses have been traditionally grown under marginal conditions of poor soil moisture, fertility and different other abiotic stresses, while the more of the fertile lands, as well as the improved agronomic practices have always been reserved for the cereal crops. These constraints have resulted in lower productivity and availability of pulses, besides significant efforts for improving the cultivars for fertile lands. One of the approaches to increase grain legume production is to expand the cultivation of different species in the adversely affected soils through developing cultivars which are more tolerant to different abiotic and biotic stresses.

Mapping populations are usually obtained from controlled crosses. Decisions on selection of parents and mating design for development of mapping population and the type of markers used depend upon the objectives of experiments, availability of markers and the molecular map. The parents of mapping populations must have sufficient variation for the traits of interest at both the DNA sequence and the phenotype level. The variation at DNA level is essential to trace the recombination events. The more DNA sequence variation exists, the easier it is to find polymorphic informative makers. When the objective is to search for genes controlling a particular trait, genetic variation of trait between parents is important. If the parents are greatly different at phenotypic level for a trait, there is a reasonable chance that genetic variation exists between the parents, although uncontrolled environmental effects could create large phenotypic variation without any genetic basis for the effects.

Blackgram or urdbean is one of the important grain legumes of India and Munhgbean yellow mosaic virus (MYMV) disease is one of the most devastating diseases. Munhgbean yellow mosaic virus (MYMV), a whitefly-transmitted bigeminvirus, causes disease in blackgram. This virus is assigned to the genus *Begomovirus* within the family *Geminiviridae*. Plant resistance is the most common and

efficient method of MYMV control. In blackgram, two symptom types yellow mosic and necrotic mottle, can be distinguished (Nair and Nene, 1974). A strategy to improve the efficiency of mapping, named selective mapping, was proposed by Visionet al. (2000). It consists of a two-step process in which, first, a mapping population of usual size (N¼60–250) is used to construct a saturated framework map with markers placed on it with high precision, and second, new markers are added to this map with lower precision using a selected subset of highly informative plants.

To attain the objectives of mapping MYMV resistance with DNA markers, it was proposed to develop F_2 population from a cross between IPU 982, an improved high yielding recommended variety but highly susceptible to MYMV infection, and Cuttack Local, a local landraces (LLRs) from the Cuttack district of Orissa known to be a low yielder but resistant to MYMV disease. The cross was done at Allahabad and the F1 progenies were also planted there. The F2 generation was grown in NBPGR, New Delhi along with the parents. But at Delhi all the F_2 individuals were found to be susceptible along with the parents except some individuals (Plant no 47, 65, 80 and 90) according to interaction phenotypic scale. Due to some unavoidable conditions and the total number of F_2 individuals was found to be very less. The climatic conditions in which the F_2 individuals were grown was not appropriate. Either due to pathogens found in Delhi or the environmental conditions the number of F2 was reduced to 46. These small number of F2 population were not sufficient to generate a molecular map. Hence our study was further extended to find the closest wild species related to our crop which are resistance to some disease so that diversity analysis and gene flow can be studied. Further we tried to search for the single nucleotides which made differences. Some more accessions were used and the main objectives of using these markers were

v) Elucidation of genetics of resistance to YMV diseases in urdbean

vi) Molecular mapping of genes controlling resistance to YMV.

vii) Studying genetic relationships among important *Vigna* cultivars and wild species using molecular markers.

iv) Study of genetic variability in different gene pools based on molecular gene sequence

DNA for this analysis extracted following the CTAB extraction protocol of Saghai-Maroof *et al.* (1984).The STMS technique was optimised for DNA quantity (40 ng), $MgCl_2$ and annealing temperatures of the primer pairs. Initially 66 RAPD primers were chosen but later on the more efficient makers were used. A total of 10 STMS and 6 STS primer pairs generating optimum and consistent PCR profiles were selected for the analyses. The DNA profiling of the 25 accessions with selected primer pairs yielded good polymorphic amplification products. The PCR products that were not consistent with the estimated allele sizes for a locus were not scored since these were considered to be non-specific amplifications.

. The data was also used to analyze the population genetic parameters such as total number of alleles, effective number of alleles, Shannon Information index etc. The maximum number of alleles found was 5 with *V. r. silvestris.* Ewens –Watterson test for neutrality was done to analyze the occurrence of selection and gene flow. Analysis of molecular variation (AMOVA) was done to find out the genetic differentiation between and within populations and among groups. Dendogram was made for the 25 accessions to show the arrangement of the accessions cluster wise.

The sequencing was done for the samples by which the numbers of segregating sites (S), observed nucleotide diversity per site between any two sequences assuming that the sample is random (p), number of haplotypes (H), haplotype diversity (Hd), and average number of pairwise nucleotide differences within population (K) were calculated using DnaSP. Tajima's D correspond to positive natural selection whereas negative values correspond to negative or purifying selection. The genetic differentiation among the parasite populations was calculated in terms of fixation index (Fst) that estimates diversity within a subpopulation with respect to total genetic diversity. In addition, average number of pairwise nucleotide differences (Kxy), nucleotide substitution per site (Dxy), and net nucleotide substitution per site (Da) between populations were also calculated. The above parameters were also estimated on DnaSP. Phylogenetic analysis

was performed by neighbour-joining (NJ) method with Kimura 2-parameter distance matrix inMEGA version 3.0.

The major conclusions drawn were that due to recent development of gene transfer technology, genes from cross incompatible wild species can be use for breeding. Therefore, evaluation of a broad array of wild species is an important approach to seeking genes which rare or absent in cultigens.

REFRENCES

Ajibade SR, Weeden NF, Chite SM (2000) Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111: 47-55.

Akagi H, Yokozeku Y, Inagaki A, Fuzimura T (1996) Microsatellite DNA markers for rice chromosomes. Theor. Appl. Genet. 93: 1071-1077.

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length Polymorphisms of simple sequence DNA in soybean. Genetics 132: 1131-1139.

Alvarez I, Wendel JF (2003) Ribosomal ITS sequences and plant phylogenetic inference. Molecular Phylogenetics and Evolution 29: 417-434.

Arcade AF, Anselin P, Rampant F, Lesage MC, Praques LE, Prat D (2000) Application of AFLP, RAPD and ISSR markers to genetic mapping of European and Japanese larch. Theor. Appl. Genet. 100: 299-307.

Arora RK, Chandel KPS, Joshi BS (1973) Morphological diversity in *Phaseolus sublobatus* Roxb. Curr. Sci. 42: 359-361.

Audilakshmi B, Chandel KPS (1990) Interspecific hybridization and crossability studies among cultivated *Vigna* species and other closely related wild taxa. Indian J. Plant Genet. Res. 3(1): 46-60.

Babu CR, Sharma SK, Chatterjee SR, Arbol YP (1988) Seed protein and amino acid composition of *V. radiata* var. *sublobata* (*Fabaceae*) and two cultigens, *V. mungo* and *V. radiata*. Eco. Bot. 42(1): 54-61.

Babu CR, Sharma SK, Johri BM (1985) Taxonomic revision of Indian *Phaseolus, Vigna, Macroptilium* and *Dysolobium*. Bull. Bot. Surv. Ind. 27: 1-28.

Baldwin BG (1992) Phylogenetic utility of internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the *Compositae*. Molecular Phylogenetics and Evolution. 1: 3-16.

Baldwin BG (1993) Molecular phylogenetics of Calycadenia (Compositae) based on ITS sequences of nuclear ribosomal DNA: Chromosomal and morphological evolution reexamined. Am. J. Bot. 80: 222-238.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Champbell CS, Donoghue MJ (1995) The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Ann. Missouri Bot. Gard. 82: 247-277.

Baudin JP, Maréchal R (1988) Taxonomy and evolution of genus *Vigna*. In: Mungbean. Proc. 2nd Int. Symp Asian Vegetable Res Dev Center. Shanhua, Taiwan, pp 2-12.

Baum DA, Smaii RL, Wendel JF (1998) Biogeography and floral evolution of baobabs (*Adansonia,* Bombacaceae) as inferred from multiple data sets. Syst. Biol. 47: 181-207.

Becker J, Heun M (1995) Mapping of digested and undigested random amplified microsatellite polymorphisms in barley. Genome 38: 991- 998.

Beckman JS, Soller M (1990) Towards a unified approach to genetic mapping of eukaryotes based on sequenced tagged micro satellite sites. Biotechnology 8: 30 - 32.

Beebe S, Toro Ch O, Gonzalez, Chacon MI, Debouck DG (1997) Wild-weed-crop groupes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Columbia, and their implications for conservation and breeding. Genet. Res. Crop Evol. 44: 73-91.

Bennett MD (1976) DNA amount, latitude and crop plant distribution. Envir. Exptl. Bot. 16: 93-108.

Bennett MD (1977) The time and duration of meiosis. In: A Discussion on the Meiotic Process. (eds Riley R, Bennett MD, Flavell RB), Phil. Trans. Roy. Soc. Ser. B. 277: 201-276.

Bennett MD (1982) Nucleotypic basis of the spatial ordering of chromosomes in eukaryotes and their implications of the order of genome evolution and phenotypic variation. In: Genome Evolution (eds Dover GA, Flavell RB), pp. 239-261. Academic press, London.

Bentham G (1865) Leguminosae. In: Genera Plantarum. Vol. 1 (eds Bentham G, Hooker JD), pp. 434–600, Lovell Reeve and Co., London.

Bisht IS, Bhat KV, Lakhanpaul S, Latha M, Jayan PK, Biswas BK, Singh AK (2005) Diversity and genetic resources of wild *Vigna* species in India. Genet. Res. Crop Evol. 115:15-25.

Biswas MR, Dana S (1976a) *Phaseolus aconitifolius* x *Phaseolus trilobus*. Indian J. Genet. 36: 125-131.

Biswas MR, Dana S (1976b) Meiosis in amphidiploid of *Phaseolus aureus* Roxb. x *P. calcaratus* Roxb. J. Soc. Expt. Agric. I: 43-44.

Blair MW, Panaud O, McCouch SR (1999) Inter simple sequence repeat (ISSR) amplification for analysis of micro satellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor. Appl. Genet. 98: 780-792.

Bose RD (1939) Studies in Plant pulses. IV Mung or Green grams (*Phaseolus aureus* Roxb.). Indian J. Agric. Sci. 2: 607-624.

Brunk CF, Jones KC, James TW (1979) Assay for nanogram quantities of DNA in cellular homogenates. Annal. Biochem. 92: 497-500.

Buckler ES, Ippolito A, Hotsford TP (1997) The evolution of ribosomal DNA: Divergent paralogs and phylogenetics implications. Genetics 145: 821-832.

Bukhari YM, Koivu K and Tigerstedt PMA (1999). Phylogenetic analysis of *Acacia* (Mimosceae) as revealed from chloroplast RFLP data. Theor. Appl.Genet. 98: 291-298.

Chandel KPS (1980) Evolutionary studies in *Vigna radiata, V. mungo* and other species. M.Sc. Thesis, Crop Evolution Laboratory, Dept. of Plant Biology Univ. Birmingham, England. pp. 1-100.

Chandel KPS, Lester RN, Starling RJ (1984) The wild ancestor of urd and mungbeans (*V. mungo* (L.) Hepper and *V. radiata* (L.) Wilczek). Bot. J. Linn. Soc. 89: 85-96.

Charters YM, Wilkinson MJ (2000) The use of self pollinated progenies as 'in-groups' for the genetic characterization of cocoa germplasm. Theor. Appl. Genet. 100: 160-166.

Chen NC, Baker LR, Homma S (1983) Interspecific crossability among four species of *Vigna* food legumes. Euphytica 32: 925-927.

Choumane W, Winter P, Baum M, Kahl (2004) Conservation of microsatellite flanking sequences in different taxa of Leguminosae. Euphytica 138 (3): 239-245.

Choumane W, Winter P, Weigand F, Kahl G (2000) Conservation and variability of sequence-tagged microsatellite sites (STMSs) from chickpea (*Cicer aerietinum* L.) within the genus *Cicer*. Theor Appl. Genet. 101: 269-278.

Cipriani G, Testolin R and Gardener R (1998) Restriction site variation of PCR-amplified chloroplast DNA regions and its implications for the evolution and taxonomy of *Actinidia*. Theor. Appl. Genet. 96: 389-396.

Coyne DP (1964) Species hybridization in *Phaseolus*. J. Heredity 55: 5-6.

Cronn RC, Cedroni M, Hselkorn T, Grover C, Wendel JF (2002) PCR-mediated recombination in amplification products derived from polyploidy cotton. Theor. Appl. Genet. 104: 482-489.

Dana S (1966a) Species cross between *Phaseolus aureus* Roxb. and *P. trilobus* Ait. Cytologia 31: 176-187.

Dana S (1966b) interspecific hybrid between *Phaseolus mungo* L. x *P. trilobus* Ait. J. Cytology and Genetics 1: 61-65.

Dana S (1966c) Cross between *Phaseolus aureus* Roxb. and *P. riccardianus* Ten. *Genetica Iberica* 18: 141-156.

Dana S (1966d) Chromosome differentiation in tetraploid *Phaseolus* Species and *P. riccardianus* Ten. Nucleus 9: 97-101.

De DN, Krishnan R (1966) Cytological studies of the hybrid *Phaseolus aureus* x *P. mungo*. Genetica 37: 588-600.

De DN, Krishnan R (1968a) Cytological studies in *Phaseolus*, I Autotetroploid *Phaseolus aureus* x tetraploid species of *Phaseolus* and the backcross. Indian J. Genet. 28: 12-22.

De DN, Krishnan R (1968b) Cytogenetical studies in *Phaseolus*, II *Phaseolus* x tetraploid *Phaseolus* species and the amphidiploid. Indian J. Genet. 28: 23-30.

Demesure B, Comps B, Petit RJ (1996). Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. Evolution 50: 2515-2520.

Demesure B, Dodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology 4: 129-131.

Doi K, Kaga A., Tomooka N, Vaughan DA. (2002) Molecular phylogeny of genus *Vigna* subgenus *Ceratotropis* based on rDNA ITS and atpB-rbcL intergenic spacer for cpDNA sequences. Genetica 114: 129-145.

Dumolin S, Demesure B, Petit RJ (1995) Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. Theor. Appl. Genet. 91: 1253-1256.

Dumolin-Lapegue S, Pemonge MH, Petit RJ (1997) An enlarge set of consensus primers for the study of organelle DNA in plants. Molecular Ecology 6: 393-397.

Edwards A, Civitello H, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am. J Hum Genet. 49: 746-756.

Edwards KJ, Baker JHA, Daly A, Jones C, Karp A (1996) Micro satellite libraries enriched for several micro satellite sequences in plants. Biotechniques 20: 75-78

Egawa Y (1988) Phylogenetic differentiation between three Asian *Vigna* species, *V. radiata, V. mungo* and *V. umbellata*. Bull. Natl. Inst. Agrobiol. Resour. 4: 189-200.

Egawa Y, Chotecheun S, Tomooka N, Lairungreang C, Nakeeraks P, Thavarasook C, Kitbamroong C (1996) Collaborative research program on mungbean germplasm (subgenus *Ceratotropis* of genus *Vigna*) between DOA Thailand and JIRCAS, Tsukuba, Japan, In: Mungbean Germplasm: Collection Evaluation and Utilization for Breeding Program (eds P. Srinives, C. Kitbamroong and S. Miyazaki), pp. 1-8. JIRCAS, Tsukuba, Japan.

Egawa Y, Nakagawara M, Fernandez GCJ, Gatehouse AMR, (1990) Cross compatibility and cytogenetical relationships among Asian *Vigna* species. In: Bruchids and legumes: Economics, Ecology and Coevolution (eds Fuzii K, Johnson CD, Micthel R, Yoshida T), pp. 201-208. Kluwer Acadamic Publishers.

Egawa Y, Tomooka N (1994) Phylogenetic differentiation of *Vigna* species in Asia. In: Plant Genetic Resources Management in the Tropics, pp. 112-120. JIRCAS International Symposium Series no.2. JIRCAS, MAFF, Tsukuba, Japan.

El Mousadik A, Petit RJ (1996). Chloroplast DNA phylogeography of the argan tree of Morocco. Molecular Ecology 5: 547-555.

Evans LT (1983) Photosynthetic activity and partitioning. In: Chemistry and World Food Supplies: The New Frontiers (eds Shemilt L.W.), pp. 621-631. ChemrawnII, Pergamon Press, New York.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction sites. Genetics 131: 479-491.

Fahima T, Rodder MS, Wendelhake K, Kirzhner VM, Nevo E (2002) Microsatellite polymorphism in natural population of wild emmer wheat (*Triticum dioccoides*) in Israel. Theor. Appl. Genet. 104: 17-29.

Farris J (1989) The retention index and the rescaled consistency index. Cladistics 5: 417-419.

Fatokun CA, Danesh D and Young ND (1993) Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. Theor. Appl. Genet. 86: 97-104.

Fineschi S, Tairchini D, Villani F, Vendramin GG (2000) Chloroplast DNA polymorphism reveals little geographic structure in *Castanea sativa* Mill. (*Fagaceae*) throughout Southern European countries. Molecular Ecology 9: 1495-1503.

Flavell RB (1982) Sequence amplification, deletion and rearrangement: Major source of variation during species divergence. In: Genomic Evolution. (eds Dover G. A. and Flavell R.B.), pp. 301-323. Academic press, London.

Ford R, Roux L, Itman K, Brouwer JB, Taylor PWJ (2002) Genome-specific sequence tagged microsatellite site (STMS) markers for diversity analysis and genotyping in *Pisum* species. Euphytica 124: 397-405.

Fosto M, Azanza Jean-Louis, Pasquet R, Raymond J (1994) Molecular heterogeneity of cowpea (*V. unguiculata Fabaceae*) seed storage proteins. Pl. Syst. Evol. 191: 39-56.

Frey W, Michael S, Kathrin M (1999) Chloroplast DNA-relationship in Palaeoaustral *Lopidium concinnum* (Hypopterygiaceae) an example of stenoevolution in Moses. Studies in Austral temperate rainforest bryophytes. Pl. Syst. Evol. 218: 67-75.

Fuller DQ (2002) Fifty years of archaeobotanical studies in India: laying a solid foundation. In: Settar S, Korisettar R eds. Indian Archeology in retrospect, volume III. Archaeology and interactive disciplines. Delhi: Manohar, 247-263.

Fuller DQ (2006) Agricultural origins and frontiers in south Asia: a working synthesis. Journal of World Prehistory 20: 1-86.

Fuller DQ and Korisettar R (2004) The vegetational context of early agriculture in South India. Man and Environment 29: 7-27.

Fuller DQ, Harvey E (2006) The archaeobotany of Indian pulses: identification, processing and evidence for cultivation. Environmental Archaeology 11: 219-246.

Fuller DQ, Korisettar R, Venkatasubbaiah PC and Jones MK (2004) Early plant domestication in southern India: some preliminary archaeobotanical results. Vegetational History and Archaeobotany 13: 115-119.

Fuzi K, Miyazaki S (1987) Infestation resistance of wild legumes *Vigna sublobata* to azukibean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidaceae) and its relationship with cytogenetic classification. Appl. Ent. Zool. 22: 229-230.

Fuzii K, Ishimoto M, Kitamura K (1989) Patterns of resistance to bean weevils (Bruchidae) in *Vigna radiata-mungo-sublobata* complex inform the breeding of new resistant varieties. Appl. Ent. Zool. 24 (1): 126-132.

Gagnepain F (1916) *Phaseolus luteolus* Gagnep., nom. nov. In: Flore Génerale de L'Indo-Chine, Vol. 2 (eds Lecomté PH, Humbert H). pp. 229, Masson, Paris. *Phaseolus luteolus* (Jacquin) Gagnepain, in Lecomte, Fl. Gen. Indochine 2: 229.

Gaitan–Solis E, Duque MC, Edwards KJ, Tohme J (2002) Microsatellite Repeats in common bean (*Phaseolus vulgaris*): isolation, characterization, and cross–species amplification in *Phaseolus* ssp. Crop sci. 42: 2128 – 2136.

Gepts P, Bliss FA (1984) Enhanced available methionine concentration associated with higher phaseolin levels in common bean seeds. Theor. Appl. Genet. 69: 47-53.

Ghafoor A, Ahmed Z, Quereshi AS, Bashir M (2002) Genetic relation in *V. mungo* (L.) Hepper and *V. radiata* (L.) Wilczek based on morphological traits and SDS-PAGE. Euphytica 123: 367-378.

Gifford RM, Evans LT (1981) Photosynthesis, carbon partitioning and yield. Ann. Rev. Plant Physiol. 32: 485-509.

Giraldo E, Viruel MA, Lopez-Corrales M, Hormaza JI (2005) Characterization and cross species transferability of microsatellite in common fig (*Ficus carica* L.). Journal of Horticultural Science & Biotechnology 80(2): 217-224.

Goel S, Raina SN, Ogihara Y (2002) Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of nuclear ribosomal DNA in the *Phaseolus-Vigna* group. Molecular Phylogenetics and Evolution 22(1): 1-19.

Gomez-Campo C (1980) Morphology and morph taxonomy of the tribe Brassiere. In: *Brassica* crops and wild allies(eds. Sundaes, Piñata, Gomez-Campo, C). pp 3-31.Japan scientific societies press, Tokyo.

Gomez-Campo C (1999) Taxonomy. In: Biology of *Brassica* cenospecies. (ed. Gomez-Campo C), pp. 3-23, Elsevier Science, Amsterdam,.

Gopinathan MC, Babu CR (1986) Meiotic studies of F_1 hybrids between rice bean (*Vigna umbellata*) and its wild relative (*V. minima*). Genetica 71: 115-117.

Gopinathan MC, Babu CR, Shivanna KR (1986) Interspecific hybridization between rice bean (*Vigna umbellata*) and its wild relative (*V. minima*): Fertility-Sterlity relationships. Euphytica 35: 1017-1022.

Govindraju DR (1988) Relationship between dispersal ability and levels of gene flow in plants. Oikos 52: 31-35.

Gupta MJ, Chyi J, Romero-Severson, Owen JL (1994) Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. Theor. Appl. Genet. 83: 998-1006.

Gupta PK, Balyan HS, Sharma PC, Ramesh B (1996) Microsatellites in plants: a new class of molecular markers. Curr. Sci. 70: 45-54.

Gupta SK, Roy SK (1985) Comparisons of meiotic abnormalities induced by gamma rays between a diploid and a species of *Physalis*. Cytologia 50: 167-175.

Hamada H, Petrino MG, Kakunaga T (1982) A novel repeat element with Z-DNA forming potential is widely found in evolutionary diverse eukaryotic genomes. Proc. Natl. Acad. Sci. U.S.A. 79: 6465-6469.

Hamilton MB, Pincus EL, DI Fiore A, Fleischer RC (1999) universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27: 500-507.

Hantula J, Dusabenyagasani M, Hamelin RC (1996) Random amplified microsatellites (RAMS)-a novel method for characterizing genetic variation within fungi. European Journal of Forestry Pathology 26: 159–166.

Hara H (1985) Critical notes on some type specimens of East-Asiatic plants in foreign herbaria. J. Jap. Bot. 30: 138-141.

Harlan JR (1965) the possible role of weed races in the evolution of cultivated plants. Euphytica 14: 173-176.

Harlan JR and DeWet JMJ (1965) Some thoughts about weeds. Econ. Bot. 19: 16-24.

Harlan JR and DeWet JMJ (1971) Towards a rational classification of cultivated plants. Taxon 20: 509-517.

Harlan, J.R., 1984.evaluation of wild relatives of crop plants. In: J.H.W. Holden and J.T. Williams (Eds.), Crop Genetic Resources: Conservation and Evaluation, pp. 212:222 George Allen and Unwin, London, U.K.

Harris SA, Ingram R (1991) Chloroplast DNA and biosystematics: the effects of intraspecific diversity and plastid transmission. Taxon 40: 393-412.

Hartl DL (1987) A primer of population genetics, 2nd edn. Sinauer Associates Inc. Publishers. Sunderland, Massachusetts.

Hillis DM, Moritz C, Porter A, Baker RJ (1991) Evidence for biased gene conversion in concerted evolution of ribosomal DNA. Science 251:308-310.

Ignacimuthu S (1988) Nuclear DNA and RNA amounts in wild and cultivated urd and mung beans and their MI mutant plants. Cytologia 53: 535-541.

Ignacimuthu S, Babu CR (1984a). Phenotypic variations in natural populations of *Vigna radiata* (L.) Wilczek var. *sublobata* (Roxb.) Verdc. (Leguminosae-Papilionoideae). Indian J. Genet. 44: 286-296.

Ignacimuthu S, Babu CR (1984b) Breeding potential of *Vigna sublobata* (Roxb.) Babu and Sharma in the improvement of mungbean. Curr. Sci. 53: 786-788.

Ignacimuthu S, Babu CR (1985) Phenotypic variation in natural populations of *V. radiata* var. *sublobata* (Roxb.) (Leguminosae-Papilionoidae). Indian J. Genet. 44: 286-296.

Ignacimuthu S, Babu CR (1987) Mutagenesis for resistance to storage pest in the wild and cultivated urd and mungbean. J. Nuclear Agric. Biol. 16: 169-176.

Ignacimuthu S, Babu CR (1989) Induced chromosomal abnormality and pollen sterility in wild and cultivated urd and mungbean. Cytologia 54: 159-167.

Isagi Y, Suhandono (1997) PCR primers amplifying microsatellite loci of *Quercus myrsinifolia* Blume. and their conservation between oak species. Molecular Ecology 6: 897-899.

Jaaska V, Jaaska V (1989) Isozyme differentiation between Asian beans *Vigna radiata* and *V. mungo*. Biochem. Physiol. Pflanzen. 185: 41-53.

Jaaska V, Jaaska V (1990a) Isoenzyme variation in the genera *Phaseolus* and *Vigna* (fabaceae) in relation to their systematics: ASPARTATE aminotransferase and superoxide dismutase. Pl. Syst. Evol. 159: 145- 159.

Jaaska V, Jaaska V (1990b) Isozyme variation in Asian beans. *Bot. Acta*. 103: 281-290.

Jaccard P (1908) Etude comparative de a Distribution florale dans portiondes Alpes et des Jura. *Bull. Soc. Vaudoise Sci. Nat.* 37: 547-579.

Jacob HJ, Linpainter K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzan VJ, Lander ES (1991) genetic mapping of a gene causing hypertension in the stroke- prone spontaneously hypertensive rat. Cell 67: 213-214.

Jain HK, KL Mehra (1980) Evolution, adaptation, relationships and uses of the species of *Vigna* cultivated in India. In: Advances in Legume Science, (eds Summerfield RJ, Bunting AH), pp. 459-468, Kew. Royal Botanic Gardens.

Jarne P, Lagoda PJL (1996) Microsatellites from molecules to population and back. Trends Ecol. Evol. 11: 424-429.

Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000) Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theor. Appl. Genet. 100: 1311–1320.

Kaga A, Tomooka N, Egawa Y, Hosaka K, Kamijima O (1996) Species relationships in the subgenus Ceratotropis (genus *Vigna*) as revealed by RAPD analysis. Euphytica 88: 17-24.

Kajale MD (1974) Plant economy at Bhokhardan, Excavation at Bhokhardan (Bhogavadana) 1973. 2, (eds Deu SB, Gupta RS). pp. 7-224, Nagpur University and Maharashtra University.

Kajale MD (1977) On the botanical findings from Excavations at Daimabad, A Chalcolithic site in Western Maharashtra, India. Curr. Sci. 46 (23): 818-819.

Kallo (1972) Chromosomal alterations in mitotic and meiotic systems as influenced by gamma rays in *Pisum*. Cytologia 37: 643-651.

Kandpal RP, Kandpal G, Weissman SM (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region specific markers. Proc. Natl. Acad. Sci. U.S.A. 91: 88-92.

Karmakar PG, Dana S (1987) Cytogenetic identification of *Vigna sublobata* collection. The Nucleus 30 (1,2): 47-53.

Kavimandan SK, Chandel KPS (1988) Nodulation in some wild species of *Vigna* and symbiotic behavior of their root nodule bacteria. New Botanist 15 (4): 253-258.

Kijas JMH, Fowler JCS, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. Biotechniques 16: 657-662.

Kim SC, Crawford DJ, Jansen RK, Santos-Guerra A (1999) The use of non-coding region of chloroplast DNA in phylogenetic studies of the subtribe Sonchinae (Asteraceae: Lactuceae). Pl. Syst. Evol. 215: 85-99

Kimura M: (1980).A simple method for estimating evolutionary rates of base substitutions through omparative studies of nucleotide sequences. *J Mol Evol*, **16**:111-120.

Kitamura K, Ishimoto M, Sawa M (1988) Inheritance of resistance to infestation with azukibean weevil in *Vigna sublobata* and successful incorporation to *V. radiata*. Japan J. Breed. 38: 459-464.

Kleynhans R, Spies JJ (2000) Evaluation of genetic variation in *Lachenalia bulbifera* (Hyacinthaceae) using RAPDs. Euphytica 115: 141-147.

Kluge A, Farris J (1969) Quantitative phyletics and evolution of anurans (1969) Syst. Zoo. 18: 1-32.

Koch MA, Matschinger M (2007) Evolution and genetic differentiation among relatives of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U.S.A. 104: 6272-6277.

Kojima T, Nagaoka T, Noda K, Ogihara Y (1998) Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. Theor. Appl. Genet. 96: 3-45.

Kress WJ, Prince LM, Williams KJ (2002) The phylogeny and new classification of gingers (Zingiberaceae): evidence from molecular data. Am. J. Bot. 89: 1682-1696.

Kumar S, Tamura K, Nei M (2008) MEGA: Molecular Evolutionary genetics Analysis software for microcomputers. Bioinformatics 10: 189-191.

Kumar S, Tamura K, Nei M: (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*, **5:**.

Ladizinsky, C., 1985. Founder effect on crop-plant evolution. Econ. Botany 39:191-199.

Lakhanpaul S (1989) Genome organization and evolution in *Vigna* Savi Ph. D. thesis, University of Delhi, Delhi, India.

Lakhanpaul S, Chadha S, Bhat KV (2000) Random amplified polymorphic DNA (RAPD) analysis in Indian mungbean [*V. radiata* (L.) Wilczek] cultivars. Genetica 109: 227-234.

Langercrantz V, Ellegra H, Anderson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nu. Acids Res. 21: 1111-1115.

Lawn R, Williams W, Imrie BC (1988) Potential of wild germplasm as a source of tolerance to environmental stresses in mungbean. In: Mungbean Proc. pp. 136-1452nd Int. Symp., AVRDC, Taiwan.

Lawn RJ (1995) The Asiatic *Vigna* species. In: The Evolution of Crop Plants (eds Smartt J, Simmonds NE), pp. 321-326. 2nd edition. Longman, Harlow, U.K.

Lench NJ, Norris A, Bailey A, Booth A, Markham AF (1996) Vectorette PCR isolation of microsatellite repeat sequences using anchored dinucleotide repeat primers. Nuc. Acids Res. 24 (11): 2190-2191.

Levin IN, Gilboa E, Yeselson S, Shen, Schaffer AA (2000) *Fgr*, a major locus that modulates the fructose to glucose ratio in mature tomato fruits. Theor. Appl. Genet. 96: 256-262.

Levinson G, Gutman GA (1987) Slipped strand mispairing: a major mechanism for DNA sequence evolution. Mol. Biol. Evol. 4: 203-221.

Lewontin R (1972) The apportionment of human diversity. Evol. Biol. 6: 381-398.

Litt M and Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet. 44: 397-401.

Lorimer GH (1981) The carboxylation and oxygenation of ribilose-1, 5-bisphosphate; the primary events in photosynthesis and respiration. Ann. Rev. Plant Physiol. 32: 349-383.

Lukoki L, Maréchal R, Otoul E (1980) The wild ancesstors of cultivated beans *V. radiata* and *V. mungo*. Bull. Jard. Bot. Nat. Belg. 28: 23-30.

Machado M, Tai W, Baker LR (1982) Cytogenetic analysis of the interspecific hybrid *Vigna radiata* x *V. umbellata*. J. Heredity 73: 205-208.

Malik M, Vyas P, Rangaswamy NS, Shivanna KR (1999) Development of two new cytoplasmic male sterile lines in *Brassica juncea* through wide hybridization. Plant Breed. 118: 75-78.

Maréchal R, Mascherpa JM, Stainier F (1978) Etude taxonomique d' un group groupe d' especes des generes *Phaseolus* et *Vigna* (Papillionaceae) sur la base de donnees morphologiques, et polliniques traitees par l' analyse informatique. Boissera 28: 1-273.

Matsouka Y, Vigouroux Y, Goodman MM, Sanchez J, Buckler E, Doebley J (2002) A single domestication for maize shown by multilocus micro satellite genotyping. Proc. Natl. Acad. Sci. USA. 99: 6080-6084.

McCree KJ (1983) Carbon balance as a function of plant size in sorghum plants. Crop Sci. 23: 1173-1177.

McDade LA (1992) Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. Evolution 46: 1329-1346.

McDade LA (1995) Hybridization and phylogenetics. In: Experimental and Molecular Approaches to Plant Biosystematics (eds Hoch PC, Stephenson AG), pp. 305-331, Missouri Botanical Gardens, St Louis, Missouri.

McGregor CE, Lambert CE, Greyling MM, Louw JH, Warnich L (2000) A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica 113: 135-144.

Meyer W, Micthell TG, Freedman EZ, Vilgalys R (1993) Hybridization probes for conventional DNA fingerprinting used as single primer in polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. Jour. Clinical Biol. 31: 2274-2280.

Mignouna HD, Ng NQ, Ikea J, Thottapilly G (1998) Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. J. Genet. Breed. 53: 151-159.

Mihardja D, Lester RN (1974) Comparative studies of some Asiatic species of *Phaseolus* using electrophoretic analysis of seed proteins. Annales Bogoreinsis 5 (4): 159-171.

Miyazaki H (1982) Classification and phylogenetic relationship of the *Vigna radiata – silvestris – sublobata* group. Bull. Nat. Ints. Sci. D. 33: 1-61.

Mohanty A, Martin JP, Aguinagalde (2001) A population genetic analysis of chloroplast DNA in wild population of *Prunus avium* L. in Europe. Heredity 87: 421-427.

Morgante M and Olivieri AM (1993) PCR amplified microsatellite as markers in plant genetics. Plant J. 3:175-182.

Mort ME, Soltis DE, Soltis PS, Francisco-Ortega J, Santos-Guerra A (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. Syst. Bot. 27: 271-288.

Nagaoka T, Ogihara Y (1997) Applicability of inter-simple sequence repeats polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94: 597-602.

Nagato Y, Yamamoto K, Yamashita H (1981) Variation of DNA content in Asian rice. Jap. J. Genet. 56: 483-493.

Naylor G, Kraus F (1995) The relationship between s and m and the retention index. Syst. Biol. 44(4): 559-562.

Nei M (1972) Genetic distance between populations. Am. Nat. 106: 283-292.

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. 76: 5269-5673.

Ogihara Y, Terachi T, Sasakuma T (1991) Molecular analysis of the hot spot region related to the length mutation in wheat Chloroplast DNAs. I nucleotide divergence of genes and intergenic spacer regions located in the hotspot region. Genetics 129: 321-332.

Oka HI, Morishima H (1971) The dynamics of plant domestication: Cultivation experiments with *Oryza perennis* and its hybrid with *O. sativa*. Evol. 25: 356-364.

Ostrander EA, Jong PM, Rine J, Duyk G (1992) Construction of small insert genomic DNA libraries highly enriched for micro satellite repeat sequences. Proc. Natl. Acad. Sci. U.S.A. 89: 3419-3423.

Otoul E, Maréchal R, Dardene G, Desmedt F (1975) Des dipeptides Soufres differencient nettement *Vigna radiata* de *Vigna mungo*. Phytochemistry 14: 173.

Ouédraogo JT, Gowda BS, Jean M, Ehlers JD, Hall AE, Gillaspie AG, Roberts PA, Ismail AM, Bruening G, Gepts P, Timko MP, Bezile FJ (2002) An improved genetic linkage map for cowpea (*Vigna unguiculata* (L.) Walp.) combining AFLP, RFLP, RAPD, biochemical markers and biological resistance traits. Genome 45: 175-188.

Palmer JD (1987) Chloroplast DNA evolution and biosystematic uses of chloroplast DNA. Am. Nat. 130: S6-S29.

Pandian A, Ford R, Taylor PWJ (2000) Transferability of sequenced tagged microsatellite sites (STMS) across four major pulses. Plant Mol. Biol. Rep. 18: 395a-395h.

Panella L, Gepts P (1992) Genetic relationships within *Vigna unguiculata* (L.) Walp. based on isozyme analyses. Genet. Res. Crop Evol. 39: 71-88.

Parani M, Lakshmi M, Ziegenhagen B, Fladung M, Senthil kumar P, Parida A (2000) Molecular phylogeny of mangroves VII. PCR–RFLP of trnS-psbC and rbcL gene regions in 24 mangrove-associated species. Theor. Appl. Genet. 100: 454-460.

Parida A, Raina SN, Narayan RK (1991) Quantitative DNA variation between and within chromosome complements of *Vigna* species (*Fabaceae*). Genetica 82: 125-133.

Pasquet RS (1999) Genetic relationships among species of *V. unguiculata* (L.) Walp. based on allozyme variation. Theor. Appl. Genet. 98: 1104-1119.

Pavliczek A, Hrda S, Flegr J (1999). Free-Tree- freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/ jackknife analysis of the tree robustness. Application in the RAPF analysis of genus *Frenkelia*. Folia Biol. 45: 97-99.

Peng JH, Fahima T, Roder MS, Li YC, Dahn A, Grama A, Ronin YI, Korol AB, Nevo E (1999) Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from

wild emmer wheat, (*Triticum dicoccoides*) and suggestive negative crossover interference on chromosome 1B. Theor. Appl. Genet. 98: 862-872.

Phansak P, Taylor PWJ, Mongkolporn O (2005) Genetic diversity in yardlong bean *Vigna unguiculata* ssp *sesquipedalis* and related *Vigna* species using sequence tagged microsatellite site analysis. Scientia Horticulturae 106 (2): 137-146.

Plitman U, Kislev ME (1989) Reproductive changes induced by domestication. In: Advances in legume biology (eds Stirton CH, Zarucchi JL), pp. 487–503, St. Louis: Missouri Botanical Garden.

Porter JM (1993a) Phylogeny of Polemoniaceae based on nuclear ribosomal internal transcribed spacer sequences. Am. J. Bot. 80 (supplement, abstract): 171.

Porter JM (1993b) Phylogenetic systematic of *Gilia* sect. *Giliandra*. Ph.D. Dissertation, University of Arizona, Tucson.

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 7: 216-222.

Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet. 98: 107-112.

Qian W, Ge S, Hong DY (2001) Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. Theor. Appl. Genet. 102: 440-449.

Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet. 9: 275-280.

Rao GU, Batra-Sarup V, Prakash S, Shivanna KR (1994) Development of a new cytoplasmic male sterile lines in *Brassica juncea* through wide hybridization. Plant Breed. 112: 171-174.

Rao SR, Chandel KPS (1991) Karyomorphological studies in the cultivated and wild *Vigna* species in Indian gene center. Cytologia 56 (1): 47-58.

Ratnaparkhe MB, Tekeoglu M, Muehlbauer FJ (1998) Inter-simple sequence repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance clusters. Theor. Appl. Genet. 97: 515-519.

Ravi V, Khurana JP, Tyagi AK, Khurana P (2008) An update on chloroplast genomes. Pl. Syst. Evol. 271, 101- 122.

Reddy MP, Sarla N, Siddiq EA (2002) Inter simple sequence repeats (ISSR) polymorphism and its application in plant breeding. Euphytica 120: 9-16.

Rees H (1984) Nuclear DNA variation and homology of chromosomes. In: Plant Biosystematics. (ed. Grant WF) pp.87-96. Academic Press, Canada.

Rees H, Jenkings G, Seal AG, Hucthinson J (1982) Assays of the phenotypic effects of changes in DNA amount. In: Genome evolution (eds. Dover G. A. and Flavell R.B.), pp. 287-297. Academic press, London.

Reeslar PM, Stucky JM, Miksche JP (1981) Cytophotometric determination of the amount of DNA in *Arachis* L. Sect. *Arachis* (*Leguminosae*). Am. J. Bot. 68: 149-153.

Richard A (1847) Tentamen Florae Abyssinicae, Arthus Bertrand, Paris.1: 216.

Riesberg LH, Brunsfeld SJ (1992) Molecular evidence and plant introgression. In: Molecular Systematics of Plants (eds Soltis PS, Doyle JJ), pp 151-176, Chapman & Hall, New York.

Riesberg LH, Soltis DE (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends in Pl Sci. 5: 65-84.

Rohlf FJ (1992) NTSYS-pc Numerical taxonomy and multivariate taxonomy system, version 1.8.

Rohlf FJ (2002) NTSYS-pc Numerical taxonomy and multivariate taxonomy system, version 2.11, Exeter software, Seteauket, New York.

Rosa R De La, James CM, Tobutt KR (2002) *Olea europaea* - Isolation and characterization of polymorphic micro satellites in Olive (L) and their transferability to other genera in the Oleaceae. Mol. Ecol. Notes 2: 265-267.

Saghai-Maroof MA, Soliman KM, Jorgenson RA, Allard AW (1984) Ribosomal spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA. 81: 8014- 8018.

Saini A, Reddy SK, Jawali N (2008) Intra-individual and intra-species heterogeneity in nuclear rDNA ITS region of *Vigna* species from subgenus *Ceratotropis*. Genet. Res. 90: 299-316.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.

Santalla M, Power JB, Davey MR (1998) Genetic diversity in mungbean germplasm revealed by RAPD markers. Plant Breed. 117: 473-478.

Savi G (1826) Troisieme memoire sur les genres Phaseolus et Dolichos par le Dr. Gaetano Savi. Linnean 1:331.

Schötterer C, Tautz D (1992) Slippage synthesis of simple sequence DNA. Nuc. Acids Res. 20: 211-215.

Sen NK, Bhowal JG (1960) Colchicine induced tetraploids of six varieties of *Vigna sinensis*. Indian J. Agric. Sci. 30: 149-161.

Sethy N K, Shokeen B, Bhatia S (2003) Isolation and characterization of sequence-tagged micro satellite site markers in chickpea (*Cicer arietinum* L.). Mol. Ecol. Notes. 3: 428-430.

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illonois Press, Urbana.

Sharma PC, Huettel B, Winter P, Kahl G, Gardener RC, Weising K (1995) The potential of micro satellite for hybridization and PCR-based DNA fingerprinting of chickpea (*Cicer arietinum* L.). Electrophoresis 16: 1755-1761.

Singh BV, Ahuja MR (1977) *Phaseolus sublobatus* Roxb. A source of resistance to yellow mosaic virus for cultivated mung. Indian J. Genet. 37: 130-132.

Slatkin M (1985) Rare alleles as indicators of gene flow. Evolution 39 (1): 53-65.

Smartt J (1980) Evolution and evolutionary problems in food legumes. Eco. Bot. 34: 219-235.

Smith JB, Bennet MD (1975) DNA variation in genus *Ranunculus*. Heredity 35: 231-239.

Soltis PS, Bloom WL (1986) Genetic variation and estimates of gene flow in *Clarkia speciosa* subsp. *polyantha* (Onagraceae). Am. J. Bot. 73 (12): 1677-1782.

Sonnante G, Piergiovanni AR, Ng NQ, Perrino P (1996) Relationships of *V. unguiculata* (L.) Walp. *V. vexillata* (L.) A. Rich and species of section *Vigna* based on isozyme variation. Genet. Res. Crop Evol. 43: 157-165.

Sonnante G, Spinosa A, Marangi A, Pignone D (1997) Isozymes and RAPD analysis of the genetic diversity within and between *V. luteola* and *V. marina*. Annals of Botany. 80: 741-746.

Sonnante G, Spinosa A, Pignone D (1998) Assessment of genetic variation in a working collection of *Vigna vexillata* (L.) Rich by isozyme and RAPD analyses. Genet. Res. Crop. Evol. 45: 347- 354.

Souframanien J, Gopalakrishna T (2004) A comparative analysis of genetic diversity in blackgram using RAPD and ISSR markers. Theor. Appl. Genet. 109: 1687-1693.

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant mol. Biol. 17: 1105-1109.

Tateishi Y (1985) A revision of Azukibean group, the subgenus *Ceratotropis* of genus *Vigna* (Leguminoseae) Ph. D. Dissertation, Tohuku University, Japan.

Tateishi Y (1996) Systematics of the species of *Vigna* subgenus *Ceratotropis*. In: Mungbean Germplasm: Collection, Evaluation and Utilization for Breeding Program (eds Srinives P, Kitbamroong C, Miyazaki S), pp. 9-24, JIRCAS, Japan.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nuc. Acids Res. 17: 6463-6471.

Tautz D and Renz M (1984) Simple sequences are ubiquitous repetitive components of Eukaryotes genomes. Nuc. Acids Res. 12: 4127- 4138.

Thompson JD, Gibson TJ, Plewniak F, Mougin FJ, Higgin DG (1997) The Clustal X Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc. Acids Res. 25: 4876-4882.

Tomooka N, Kashiwaba K, Vaughan DA, Ishimoto M, Egawa Y (2000) The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetles in the genus *Vigna* subgenus *Ceratotropis*. Euphytica. 115: 27–41.

Tomooka N, Lairingreang C, Nakeeraks P, Egawa Y, Thavarasook C (1992) Center of genetic diversity and dissemination pathways in mungbean deduced from seed protein electrophoresis. Theor. Appl. Genet. 83: 289-293.

Tomooka N, Vaughan DA, Moss H, Mixted N (2003) The Asian *Vigna*: genus *Vigna* subgenus *Ceratotropis* genetic resources New York: Kluwer.

Tomooka N, Yoon Mun Sup, Doi K, Kaga A, Vaughan D (2002) AFLP analysis of diploid species in the genus *Vigna* subgenus *Ceratotropis*. Genet. Res. Crop Evol. 49: 521-530.

Tsumura Y, Ohba K, Strauss SH (1996) Diversity and inheritance of inter-simple sequence repeat polymorphism in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theor. Appl. Genet. 92: 40-45.

Tsumura Y, Yoshimura K, Tumaru N, Ohba K (1995) Molecular phylogeny of conifers using RFLP analysis of PCR amplified specific chloroplast genes. Theor. Appl. Genet. 91: 1222-1232.

Udupa SM, Baum M (2001) High mutation rate and mutational bias at micro satellite loci in chickpea (*Cicer arietinum* L.). Mol Gen Genet. 261: 354-363.

Vaillancourt RE, Weeden NF (1993) Lack of isozyme similarity between *Vigna unguiculata* and other species of genus *Vigna* (*Leguminosae*). Can. J. Bot. 71: 586-591.

Vaillancourt RE, Weeden NF, Bruneau A, Doyle JJ (1993) Chloroplast DNA phylogeny of old world *Vigna* (Leguminosae). Sys. Bot. 18: 642-651.

Verdcourt B (1970) Studies in the Leguminosae-Papilionoidae the 'Flora of Tropical East Africa'. Kew Bull. 24: 507-569.

Virk PS, Zhu J, Newbury HJ, Bryan GJ, Jackson MT, Ford-Lloyd BV (2000) Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*Oryza sativa*) germplasm. Euphytica 112: 275-284.

Von Bothmer R. and O. Seberb, 1995.Stratigies for the collecting of wild species In: L. Guarino, V. Ramanatha Rao and R. Reid (Eds), Collecting Plant Genetic Diversity, pp. 93:111. CAB International, Willingford. U.K.

Vos P, Hogers R, Bleeker R, Reijans M, VandeLee T, HomesM, Frijters A, Pot P, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nuc. Acids Res. 23: 4407-4414.

Wagstaff JF, Wege J (2002) Pattern of diversification in New Zealand Stylidiaceae. Am. J. Bot. 89: 1086-1096.

Wang G, Mahalingam R, Karp HT (1998) (C-A) and (g-A) ancored simple sequence repeats (ASSRs) generated polymorphism in soybean *Glycine max* L. Merr. Theor. Appl. Genet. 96: 352-360.

Welsh J, McClelland (1990) Fingerprinting genomes using PCR with arbitrary primer Nuc. Acids Res. 18: 7213-7218.

Wen CS, Hsiao JY (1999) Genetic differentiation of *Lilium longiflorum* Thunb. var. *scabrum* Masam (lileaceae) in Taiwan using RAPD and morphological characters. Bot. Bull. Acad. Sin. 40: 65-71.

Wendel JF, Schnabel A, Seelanan T (1995) Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc. Natl. Acad. Sci. U.S.A. 92: 280-284.

White G, Powell W (1997) Cross- species amplification of SSR loci in the Meliaceae family. Molecular Ecology 6: 1195- 1197.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Acids Res. 18:6531-6535.

Winfeild MO, Arnold GM, Cooper F, Le-Ray M, White J, Karp A (1998) A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the upper seven areas of UK using AFLP markers. Mol. Ecol. 7: 176-183.

Wolf EC (1985) Conserving Biological diversity. In: State of the World (ed. Starke L), pp. 124-146, WW Norton and company, New York.

Wolff K, Zietkiewicz E, Hofstra H (1995). Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. Theor. Appl. Genet. 91: 439-447.

Wolff S (1981) Sister chromatid exchanges and mutagenic carcinogens. GANN Monograph Cancer Res. 27: 109–124.

Wright S (1951) The genetical structure of populations. Ann. Eugen. 15: 323-354.

Wright S (1965) The interpretation of population structure by F-statistics with special regard to system. Evolution 19: 395-420.

Wu K, Jones R, Danneberger L, Scolnik PA (1994) Detection of micro satellite polymorphisms without cloning. Nuc. Acids Res. 22: 3257-3258.

Xu, R. Q., N. Tomooka, D.A. Vaughan and K. Doi, 2000. The Vigna angularis Complex: genetic variation and relationship revealed by RAPD analysis , and their implications for in-situ conservation and domestication. Genet Res Crop Evol 46: (in press).

Yamaguchi H (1992) Wild and weed azukibeans in Japan. Econ. Bot. 46:384-394.

Yamauchi M, Yoshida S (1985) Heterosis in net photosynthetic rate, leaf area tillering and some physiological characters of 35 F_1 rice hybrids. J. Exp. Bot. 36: 247-280.

Yang W, DeOliveira AC, Godwin I, Schertz K, Bennetzen JL (1996) Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. Crop Sci. 36: 1669-1676.

Ziekiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176-183.

Zimmer EA, Roalson EH, Skog LE, Boggan JK, Indurm A (2002) Phylogenetic relationships in the Gesnerioideae (Gesneriaceae) based on nrDNa ITS and cpDNA *trnL-F* and *trnE-T*spacer region region sequences. Am. J. Bot. 89: 296-311.

Zohary D, Hopf M. Domestication of plants in the Old World (2000) 3rd edn. Oxford: Oxford University Press.

Zucchi MI, Brondani RPV, Pinheiro JB, Brondani C, Vencovsky R (2002) Transferability of microsatellite markers from *Eucalyptus* spp. to *Eugenia dysentrica* (Myrtaceae family). Mol. Ecol. Notes 2: 512-513.

ANNEXURE

ANNEXURE-I

1. Solutions, chemicals and reagents used for DNA extraction

1. Liquid Nitrogen
2. Cetyl Trimethyl Ammonium Bromide (CTAB) Buffer for DNA extraction:

(i) CTAB (10%)

10g CTAB was dissolved in sterile distilled H_2O and volume was made upto 100 ml with distilled water.

(ii) Sodium chloride (NaCl, 4M)

292.2g of NaCl was dissolved in distilled H_2O and volume was made upto 100 ml. The solution was autoclaved prior to use.

(iii) Tris: Cl buffer (pH 8.0, 1M)

12.11 g of Tris salt was dissolved in distilled H_2O and volume was made upto 100 ml and pH was adjusted to 8.0 using 1 N HCl. The solution was autoclaved prior to use.

(iv) Ethylene Diamine Tetra Acetic acid (EDTA, 0.5 M)

18.62 g EDTA was dissolved in sterile distilled H_2O. The pH of the solution was adjusted to 8.0 using IN NaOH. The volume was made upto 100 ml using sterile distilled H_2O and the solution was autoclaved.

(v) 2-Mercaptoethanol (2%)

2% solution provided by manufacturer was used directly.

Buffer composition

Component	Stock sol.	Working buffer	Vol. of stock taken to prepare 200 ml buffer
1. CTAB	10%	1.5%	40 ml
2. NaCl	4M	1.4 M	70 ml
3. Tris	1M	100mM	20 ml
4. EDTA	0.5 M	20 mM	8 ml
5. Mercaptoethanol	2%	2%	4 ml
6. Distilled H_2O	-	-	58 ml
Total			200 ml

3. Isopropanol
4. Sodium Acetate solution (3M, pH 5.6)

   30.75 g sodium acetate was dissolved in sterile distilled H_2O, pH was adjusted to 5.6 with glacial acetic acid and volume made upto 50 ml. The solution was autoclaved and stored till use.
5. Chloroform: Isoamyl alcohol (24:1) mixture

   96 ml of chloroform was mixed with 4 ml of isoamyl alcohol. It was stored in amber coloured bottle.
6. 70% Ethanol

   70 ml of absolute ethanol was mixed well with 30ml of sterile water and stored in a stoppered bottle till use.

II. DNA purification

1. Phenol: Chloroform: isoamyl alcohol (25:24:1) mixture

100 ml of Tris saturated phenol was added to a mixture of 96 ml chloroform and 4 ml isoamyl alcohol. The mixture was mixed well prior to use and stored in amber coloured bottle.

2. RNaseA (20 mg/ml) solution

RNaseA	:	20 mg
Tris-Cl (pH 7.5)	:	10mM
NaCl	:	15mM

Sterile water was added to make the volume to 1 ml. The solution was heated at 100°C for 15 minutes to inactivate any DNase present and then stored in aliquotes at -20°C.

3. Pronase (10 mg/ml) solution prepared in sterile distilled water.

III. Solvent for DNA

Tris: EDTA (TE) buffer (10 mM Tris: 1mM EDTA, pH 8.0)

10 ml of Tris (1M) buffer, pH 8.0 and 0.2 ml of 0.5 M EDTA, pH 8.0 was mixed with sterile distilled H_2O and volume made upto 100 ml. The solution was autoclaved prior to use.

IV DNA Quantification

1. **Hoechst Dye (H-33258) 10 X solution:**

10 mg of Hoechst 33258 dye was dissolved in sterile distilled H_2O and volume made upto 100 ml and stored in an amber coloured bottle at 4°C.

2. **10 X TNE buffer stock solution** (100 mM Tris: 10mM EDTA: 2M NaCl, pH 7.4)

12.11 g Tris, 3.72 g EDTA and 116.89 g of NaCl were dissolved in sterile distilled water and volume was made upto 100 ml using distilled water. The pH was adjusted to 7.4 with conc. HCl solution, filtered before use and stored at 4°C.

3. **Assay solution**

Component	Low range DNA assay 10-500 ng/ml final DNA conc.	High range DNA assay 100-5000 ng/ml
Hoechst 33258 stock soln.	10 µl	100 µl
10 X TNE	10 ml	10 ml
Distilled H_2O	90 ml	90 ml

Prepared fresh each time

4. **DNA standard**

i. **Low range assay:**

1 µl/ml of calf thymus DNA standard was used at 1:10 dilution (100 µg/ml). 2ml of calf thymus DNA (100 µg/ml) was mixed with 2ml assay solution for low range which gave 100 ng/ml standard solution.

ii. **High range assay**

2µl calf thymus DNA standard (1µg/ml) was mixed in 2 ml assay solution for high range assay gave 1000 ng/ml standard solution.

V. GEL ELECTROPHORESIS

1. **Agarose gel (1.8%)**

4.5 g agarose was added to 250 ml with 1 X TAE buffer, the contents were mixed thoroughly and boiled for 2-5 minutes to dissolve the contents. The mixture was cooled down to 40°C. The molten gel was cast in a gel tray with a comb containing 33 teeth to produce wells.

2. Ethidium bromide (10 mg ml^{-1})

10 mg of ethidium bromide was dissolved in sterile water and volume made up to 1 ml. The solution was stored in an amber coloured bottle, at 4°C.

3. Loading dye (10X) solution

1. Bromophenol Blue	0.25%
2. Xylene cyanol FF	0.25%
3. Glycerol	50%
4. TAE	1 X

4. Tris: Acetate: EDTA (TAE) buffer – 50 X (stock) solution. PH 8.0

2M tris-acetate, pH 8.0

0.05M EDTA, pH 8.0

VI. PCR COCKTAIL

1. *Taq* DNA Polymerase

A stock solution of 3 units μl^{-1} was provided by the manufacturer (Bangalore Genei) was stored at –20°C.

2. 10 X Assay buffer

10 X PCR assay buffer for *Taq* DNA polymerase containing 15mM μl^{-1} magnesium chloride provided by the manufacturer (Bangalore Genei) was used. Storage was at –20°C.

3. Deoxyribonucleotide Triose phosphate

dATP (10 mM), dGTP (10 mM), dCTP (10 mM) and dTTP (10 mM) were mixed in equal volumes and stored at –20°C till use.

4. Magnesium chloride

A solution of 15 mM μl^{-1} provided by the manufacturer, stored at –20°C, was used.

5. Primer

The primer was provided by the manufacturer in a lyophilized form. Based on the molecular weight of a given primer, a solution of 6 µM was prepared by adding the required amount of sterile water. Storage was at –20°C.

Chemicals and Materials used

Type	Material	Source
Molecular weight markers	100bp DNA ladder 1Kb DNA ladder	Fermentas Fermentas
Modifying enzymes	Taq DNA Polymerase RNase	Fermentas, Applied Biosystematics NEB Qiagen
Kits used	Dneasy DNA Extraction kit PCR product purification kit Agarose gel DNA purification kit	Qiagen Bangalore Genei Himedia
Dyes	Ethidium Bromide Xylene cyanol Methylene blue	Amersco Sigma Sigma
Fine chemicals	APS, EDTA, CTAB, Acrylamide, Bis- Acrylamide, TEMED, Agarose	Sigma, Amersco
Locally available chemicals	Isopropanol, Iso-amyl alcohol, $CaCl_2$, NaCl, NaOH, Glucose, Methanol, $MgCl_2$, Chloroform, Glycerol, Acetic acid, $MgSO_4$, Formaldehyde, β-Mercaptoethanol, Tris, Ethanol	Qualigens, Sigma, SRL

ANNEXURE-II

Table 5. Allele frequency of 25 vigna populations.

Population	Allele/ Locus	AB128093	MB122A	AB128113	AB128135	MB91	VM22	VM24	VM27	VM31	MB323b
Population: 1 Mungo Cv BBD-08-01B	Allele A	1.00	0.50		0.07		1.00	1.00	0.93	1.00	1.00
	Allele B		0.50	1.00	0.93	1.00					
	Allele D								0.07		
Population:2 Mungo Cv BBD-14-01B	Allele A	1.00	0.27				1.00	1.00	0.60	1.00	1.00
	Allele B		0.73	1.00	1.00	1.00			0.40		
Population:3 Mungo Cv BB-03-09	Allele A	1.00		0.07			1.00	1.00	1.00	1.00	1.00
	Allele B		1.00	0.93	1.00	1.00					
Population:4 V.Hyniana BB-07-01A	Allele A	1.00				1.00	1.00		1.00	1.00	1.00
	Allele B		1.00		1.00			1.00			1.00
	Allele C			1.00							
Population:5 V.Hyniana BBD-15-01A	Allele A	1.00				0.67	1.00		0.93	1.00	0.53
	Allele B		1.00		1.00	0.33		1.00	0.07		0.20
	Allele C			1.00							0.27
Population:6 Mungo Cv BB_03-10	Allele A	1.00	0.50				1.00	1.00	0.93	1.00	0.33
	Allele B		0.50	1.00	1.00	1.00					
	Allele C										0.67
	Allele D								0.07		
Population:7 v.Silvistris BBL-63-2K	Allele A	1.00	0.50	0.27			0.93	1.00	1.00	1.00	0.20
	Allele B		0.50	0.33	1.00	1.00	0.07				0.80
	Allele C			0.40							
Population:8 v.Silvistris BBL-76-2000	Allele A	1.00	0.50	0.07			0.93	1.00	1.00	1.00	
	Allele B		0.50	0.73	0.73	1.00	0.07				1.00
	Allele C			0.20	0.27						
Population:9 v.Silvistris BBL-80-2K	Allele A	1.00	0.50				1.00	1.00	0.80	1.00	0.07
	Allele		0.50	0.47	0.87	1.00			0.07		0.73

	B										
	Allele C			0.53	0.13						0.20
	Allele D									0.13	
Population:10 V.Mungo IPU-99/10	Allele A	1.00	0.50				1.00	1.00	1.00	1.00	1.00
	Allele B		0.50	1.00	1.00	1.00					
Population:11 v.Silvestris BBL-50-2K	Allele A	1.00	0.50			0.07	1.00	1.00	0.80	1.00	0.20
	Allele B		0.50	0.07	1.00	0.93					0.53
	Allele C			0.80							0.27
	Allele D			0.13							
	Allele E								0.20		
Population:12 V.Silvestris BBL-40-2K	Allele A	1.00	0.50				1.00	1.00	1.00	0.80	
	Allele B		0.50	0.13	0.87	1.00				0.20	1.00
	Allele C			0.87	0.13						
Population:13 V.Mungo Vamban 2	Allele A	0.93	0.50				1.00	1.00	0.67	1.00	1.00
	Allele B	0.07	0.50	1.00	1.00	1.00					
	Allele C								0.20		
	Allele D								0.13		
Population:14 V.Mungo PDU-1	Allele A	0.93	0.50				1.00	1.00	0.87	1.00	1.00
	Allele B	0.07	0.50	1.00	1.00	1.00			0.07		
	Allele C			0.80							0.27
	Allele D			0.13							
Population:15 V.Mungo TMV-1	Allele A	1.00	0.50				1.00	1.00	1.00	1.00	1.00
	Allele B		0.50	1.00	1.00	1.00					
Population:16 V.Mungo PDU-1016	Allele A	1.00	0.50				1.00	1.00		1.00	1.00
	Allele B		0.50	1.00	1.00	1.00			1.00		
Population:17 V.Mungo UG-414	Allele A	1.00	0.50				1.00	1.00	0.20	1.00	1.00
	Allele B		0.50	1.00	1.00	1.00			0.80		
Population:18 V.Silvestris BBL-55-2K	Allele A	1.00	0.50				1.00	1.00	0.13	1.00	
	Allele B		0.50	1.00	0.80	1.00			0.87		1.00
	Allele				0.20						

	C										
Population:19 V.Hyniana KP3-Rajgarh	Allele A	1.00				1.00	1.00			1.00	
	Allele B		0.50					1.00			0.60
	Allele C		0.50	1.00	1.00				1.00		0.40
Population:20 V.Silvestris IC-251-407	Allele A					1.00	1.00	1.00		1.00	
	Allele B	1.00	0.50	1.00	1.00				1.00		
	Allele C		0.50								
	Allele D										1.00
Population:21 V.Hyniana IC-251-377	Allele A	1.00		0.50		1.00	1.00	1.00		1.00	
	Allele B		0.50	0.50							
	Allele C		0.50		1.00				1.00		1.00
Population:22 V.Silvestris BB-2638	Allele A	0.73	0.13			0.80	1.00	1.00		0.73	
	Allele B	0.27	0.70	1.00	1.00	0.20			1.00	0.27	0.03
	Allele C		0.17								0.97
Population:23 V.Silvestris BB-2641	Allele A	0.47	0.50			1.00	0.87	1.00		0.47	
	Allele B	0.53	0.50	0.73	1.00		0.13		1.00	0.53	0.97
	Allele C			0.27							
	Allele D										0.03
Population:24 V.Silvestris BB-2642	Allele A	0.73	0.37		0.27	0.80	0.67	1.00		1.00	0.07
	Allele B	0.27	0.63	1.00	0.73	0.20	0.07		0.53		0.60
	Allele C						0.27		0.47		
	Allele D										0.33
Population:25 V.Hyniana BB-2649	Allele A	1.00			1.00	1.00	1.00			1.00	1.00
	Allele B							1.00	0.47		
	Allele C		1.00	1.00					0.47		
	Allele D								0.07		

Table Allele frequency of three groups of *vigna*.

Group	Allele/ Locus	AB128093	MB122 A	AB1281 13	AB128135	MB91	VM22	VM24	VM27	VM31	MB323b

Group 1 V.Mungo	Allele A	0.99	0.43		0.01		1.00	1.00	0.72	1.00	0.93
	Allele B	0.01	0.57	1.00	0.99	1.00			0.23		
	Allele C								0.02		0.07
	Allele D								0.03		
Group 2 V.Silvestris	Allele A	0.79	0.40	0.03	0.03	0.37	0.94	1.00	0.47	0.90	0.05
	Allele B	0.21	0.53	0.65	0.90	0.63	0.03		0.45	0.10	0.67
	Allele C		0.07	0.31	0.07		0.03		0.05		0.05
	Allele D			0.01					0.01		0.23
	Allele E								0.02		
Group 3 V.Hyniana	Allele A	1.00	0.60	0.10	0.20	0.93	1.00	0.20	0.39	1.00	0.31
	Allele B		0.40	0.10	0.40	0.07		0.80	0.11		0.36
	Allele C			0.80	0.40				0.49		0.33
	Allele D								0.01		